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Proton Nuclear Magnetic Resonance Spectroscopy (NMR) Methods for Determining the Purity of Reference Drug Standards and Illicit Forensic Drug Seizures

ABSTRACT: A rapid, sensitive, accurate, precise, reproducible, and versatile method for determining the purity of reference drug standards and the routine analysis of illicit drugs and adulterants using proton (^{1}H) Nuclear Magnetic Resonance (NMR) Spectroscopy is presented.

The methodology uses a weighed sample dissolved in a deuterated solvent or solvent mixture containing a high purity internal standard. The NMR experiment employs 8 scans using a 45 second delay and 90° pulse. In the determination of purity of reference standards, the number of quantitative determinations available is equal to the number of peak groups that are baseline resolved. The relative standard deviation (RSD) of these signals is usually $<1\%$ for pure standards, and the results agree well with other purity determining methods. This method can also aid in the determination of correct molecular weight for standards containing an unknown number of waters of hydration or an unknown number of acids per drug in salts.

Because the molar response for the hydrogen nucleus is 1 for all compounds, and since no separation media are used, only one linearity study is required to test a probe. In the presented study, the linearity of the NMR probe was determined using methamphetamine HCl dissolved in deuterium oxide (D_2O) with maleic acid as the internal standard (5 mg) for a range of concentrations from 0.033 to 69.18 mg/ml with a resulting correlation coefficient of >0.9999 for all 6 methamphetamine peak groups.

The spectra of complex illicit heroin, methamphetamine, MDMA, and cocaine samples are presented, as well as an extensive list of compounds, their solubilities and the solvent(s) and internal standard used.

KEYWORDS: forensic science, nuclear magnetic resonance spectroscopy, NMR, quantitative, drug analysis, reference standard authentication

Nuclear Magnetic Resonance (NMR) spectrometers are one of forensic sciences' most versatile instruments. Its great ability to perform identification and structure elucidation of organic molecules is well established (1,2). In addition, NMR represents a unique methodology for performing quantitation. Beginning in the mid-1950's, papers were written about NMR as a quantitative instrument for natural product, forensic, and pharmaceutical sciences (1,7). An excellent review paper by Pauli, Jaki, and Lankin (2) contains almost 200 references on the subject of quantitative NMR, focusing on natural products. However, with the great advances in NMR technology making it a powerful structure elucidation technique, and the development and improvement of quantitative separation analyses such as gas chromatography (GC) (8,9), high performance liquid chromatography (HPLC) (10,11), and capillary electrophoresis (CE) (12,13), quantitative drug analysis by NMR in forensic drug analysis decreased (as was seen by fewer and fewer papers being presented). However, NMR still has many advantages over GC, HPLC, and CE, and modern instruments can rival chromatographic techniques in specificity, speed, accuracy, precision, and flexibility.

Unlike the chromatographic techniques, NMR does not require a high purity reference standard for accurate quantitation of the target compound. This is because the functional group being observed (the nucleus of a hydrogen atom) has a molar response coefficient of 1 regardless of the compound, assuming the hydrogen will not exchange with the deuteriums of the solvent. This paper will focus on the internal standard method of quantitative proton NMR. In this method, the integral of the analyte peak(s) is (are) compared with that of the integral of a pure internal reference material peak(s). This is extremely important when a reference drug standard is not available or is extremely costly, as is commonly the case of a new "designer" drug. NMR can be used in the identification and purity determination of reference standards as well.

Another advantage of NMR is that it does not have a medium (i.e., column), which can lead to solute adsorption effects and imprecision of analysis. This can be a shortcoming in very complex mixtures, because all the signals of all the compounds in solution are present. However, such samples are unusual, and with modern NMRs of sufficient magnetic field strength ($\geq$300 MHz proton), finding signals that are free of interfering signals is usually not difficult. In addition, because there is no adsorption, there is never a need to run a "blank" sample; saving more time. Finally, because most compounds have more than one signal, and the molar response coefficient of hydrogen is 1, subtraction of interfering signals is practical and easily accomplished, resulting in additional and/or conformational quantitative results.

Finally, a wide variety of deuterated solvents are available, enabling quantitation of a large variety of drugs: basic drugs and their salts (i.e., heroin, methamphetamine, MDMA, cocaine), acidic drugs (i.e., the barbiturates and their salts), and neutral drugs (i.e., anabolic steroids). So long as the drug of interest is fully soluble, does not interact with the solvent or internal standard, the internal standard is free of interfering signals, and the compound contains non-labile hydrogens, an accurate quantitation is possible.

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The use of different solvents also expands the versatility of the methodology; for example, peaks that are overlapping in $CDCl_3$ may be well resolved in CD_3OD.

This paper describes the use of NMR for determining the purity of reference drug standards and for quantitation of illicit seized drugs.

Experimental

Instrumentation

A Varian Mercury 400 MHz NMR with an actively shielded Oxford superconducting magnet, a 23 channel room temperature shim set, a 50 position SMS auto sampler system, and a Varian Nalorac 5 mm indirect detection, variable temperature probe with PulseTune™ automatic tuning module (Varian NMR Systems, Palo Alto, CA), was used. The quantitative proton experiments had a 2-min delay to achieve thermal equilibrium in the sample, followed by automatic probe tuning, deuterium gradient shimming, and auto lock. The quantitative pulse sequence is composed of 8 scans (no steady state scans) using a 45 sec delay, 90°pulse (5 ms), and a 5 sec acquisition time (64000 points collected). The spectral window was set to −3 to 13 PPM with oversampling of 4, with inline digital signal processing (DSP) using a "brickwall" filter. The sample temperature in the probe was maintained at 25°C. A Fourier transform with zero filling to 128 K and no weighting functions were used to process the free induction decay (FID) signal. Phasing, baseline correction, and integration of commonly encountered compounds are performed automatically by macros written in-house using Varian's Magical-II® programming language. Spectra presented in this paper were created using ChemSketch, and deconvolution was performed using 1D NMR SpecManager software (version 8) by Advanced Chemistry Development Inc. (ACD/Labs, Toronto, Canada).

Materials

Maleic acid was obtained from Fluka Chemie (Buchs, Switzerland), and methenamine and dimethylsulfone from Aldrich Chemical Company (Milwaukee, WI). These were stored under vacuum in a desiccator. The following solvents and reference materials were also obtained from Aldrich Chemical Company (Milwaukee, WI): deuterium oxide, deuterium oxide containing 0.05% (by weight) 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt (TSP) as a 0 PPM reference, chloroform-d, chloroform-d containing 0.03% v/v tetramethylsilane (TMS) as 0 PPM reference, methanol-d_4, and tetramethylsilane. Reference drug standards were obtained from the Special Testing and Research Laboratory reference drug collection.

Internal Standard Solution Preparation

An internal standard could be individually weighed for each sample, but for a high volume of samples it is far easier and faster to make an internal standard solution and dispense it to the sample with a calibrated pipette. For water-soluble compounds, D_2O containing 0.05% w/w TSP is prepared with maleic acid (internal standard) to a concentration of 5 mg/mL. The higher concentrations of TSP in D_2O should not be used, since this will require lower receiver gains (lowering sensitivity) and can cause precipitation of compounds. In our experience, maleic acid in D_2O will last over a month at room temperature in a stoppered flask without changes to its concentration. Because of high usage of this internal standard solution, 500.0 mL is prepared at a time.

For chloroform soluble compounds, $CDCl_3$ containing 0.03% v/v TMS is prepared with methenamine (internal standard) to a concentration of 1 mg/mL, or dimethylsulfone (internal standard) to a concentration of 2 mg/mL. This solution is good for at least the day of use and must be kept in a sealed container due to the volatility of the chloroform and the TMS. Be aware that dimethylsulfone is a common adulterant in illicit samples (especially methamphetamine), and should be used only when peaks are not present in that region of the sample spectrum.

For methanol soluble compounds, TMS is added to methanol-d_4 to obtain approximately 0.05% v/v, and either maleic acid (5 mg/mL) or dimethylsulfone (2 mg/mL) is added as the internal standard. This solution is good for at least the day of use and must be kept in a sealed container due to the volatility of the methanol and the TMS.

As part of good laboratory practices, the newly made solution is checked against a high purity reference standard appropriate for the solvent and internal standard (i.e., dimethylsulfone for a maleic acid/D_2O internal standard solution). Experimental purities of 98-102% are acceptable. In addition, this sample is used to determine the ratio of integrals of the internal standard to the 0 PPM reference compound (i.e., TMS or TSP). This ratio should be monitored in samples to help indicate interfering peaks in the internal standard integral.

Sample Preparation

For water or methanol soluble compounds, 1.0 mL of the respective (D_2O or CD_3OD) internal standard solution is added to an accurately weighed sample (approximately 30 mg/mL) and mixed well. Should insolubles be visible in the solution, an additional 1 mL of solvent which does not contain internal standard or reference material (i.e., TMS or TSP) is added, mixed, and sonicated for 15 min. If insolubles persist, the sample is filtered.

For chloroform soluble compounds, 2.0 mL of the $CDCl_3$-methenamine-TMS internal standard solution are added to an accurately weighed sample and mixed. Should insolubles be visible, the sample is sonicated for 15 min (30 min for drugs incorporated in plastics). If insolubles persist, the sample is filtered. Earlier work used TMS alone as the internal standard yielding good agreement with GC results. Because of the volatility of TMS, special care must be taken, such as adding TMS solution after sonication. Finding a non-volatile internal standard is preferable.

A final sample concentration of 10–30 mg/mL is desirable for identification and quantitation of compounds at or below 1%. Higher concentrations are acceptable if within solubility limits. If necessary, other deuterated solvents can be added to the above solutions to enhance the solubilities of the compounds of interest. However, this can cause compound peaks to shift to new locations in the spectrum. (Note: Methanol-d_4 produces a multiplet at approximately 3.4 PPM and singlet at 4.5 PPM. In the case of $CDCl_3$/methanol-d_4 mixed solutions, the 4.5 PPM singlet can interfere with the integral of the methenamine, requiring use of another internal standard).

The equation for internal standard quantitation is as follows:

$$\%\ \text{compound in sample} = (MW_a/MW_{is}) \times (WT_{is}/WT_{samp}) \times (H_{is}/H_a) \times (INT_a/INT_{is}) \times 100$$

MW_a = molecular weight of drug
MW_{is} = molecular weight of internal standard
WT_{is} = internal standard weight (milligrams)
WT_{samp} = sample weight (milligrams)
H_{is} = number of hydrogens integrated of the internal standard
H_a = number of hydrogens integrated of the drug being quantitated
INT_a = integral of the drug peak
INT_{is} = integral of the internal standard peak

Quantitative Performance

Uniformity of response over the spectral width was tested using a single peak solution (i.e., dimethylsulfone in $CDCl_3$ or a "doped" D_2O test sample), by setting the transmitter offset to various frequencies (to cause the peak to move from one end of the spectrum to the other), acquiring one scan and having a delay of 30 sec or more between experiments. A relative standard deviation (RSD) of 0.3% for peak heights was determined in the region −0.6 PPM to +11.4 PPM, confirming that the filters used to suppress fold-in noise do not decrease signals in this region of the spectrum (which would lower quantitation results).

After sample insertion, it was determined that a 2 min delay before deuterium gradient shimming enabled thermal equilibration of the sample, thereby optimizing peak resolution. This was determined by gradient shimming and acquiring a 1 scan spectrum at 0, 1, 2, 3, and 4 min after the sample (containing dimethylsulfone and maleic acid in D_2O with TSP) was placed in the probe. The respective resolutions of the solvent, TSP, dimethylsulfone, and maleic acid peaks were determined for each experiment. At 0 and 1 min, the HDO and TSP peaks had good resolution, however, the dimethylsulfone and maleic acid peak shapes were irregular and asymmetric, and line widths were broad. However, at 2 min or more, thermal equilibrium was achieved, and all peaks in the spectrum displayed symmetry and minimal line widths.

Methamphetamine HCl in D_2O with maleic acid and TSP was used to determine the linearity, reproducibility, precision, and sensitivity limits of the probe. Methamphetamine contains signals from 1.3 to 7.5 PPM, and can therefore be used to further confirm the uniformity of response throughout the spectral width for quantitatable hydrogens. Correlation coefficients of 0.99995–1.00000 were obtained for all 6 integrals for the sample range 0.067–69.18 mg/mL, using 13 different concentrations. Five measurements were taken over a 3 day period; RSDs of the two methyls and the phenyl region (normalized to maleic acid) were <1.0%, and purities of 99.4% (RSD of 0.5%) were obtained for the same concentration range. Concentrations below 0.067 mg/mL have $S/N < 10$ when 8 scans or fewer are performed. The other signals represent one hydrogen and have multiple couplings, reducing their $S/N < 10$ at 0.33 mg/mL and below.

The solubility of a compound is determined by dissolving its high purity standard at or above the upper limit of the method's concentration (i.e., 30 mg/mL). The solution is examined visually for undissolved particles. If insolubles are present, the sample is sonicated for 15 min and vortexed. A quantitative NMR experiment is performed and the result is compared to the standard's purity (normally >98%). Results below the standard's purity indicate that the solubility limit has been exceeded. In such cases, the actual solubility concentration is determined by multiplying the quantitation results by the weight of the sample divided by the volume of the solvent (i.e., $90.0\% \times 30.0\ \text{mg}/1.0\ \text{mL} = 27\ \text{mg/mL}$ solubility limit). Once this has been determined, another sample of the standard is prepared with a concentration below the calculated solubility limit and a quantitative NMR experiment is performed on it. The results of this experiment will reflect the purity of the standard because all of the standard will be in solution (Table 1).

Compound stability is important for accurate quantitative results. Standards are run immediately after preparation and then 2 or more hours later. Results for all signals are compared to look for decomposition or deuterium exchange (Table 1).

Peak purity is determined by comparison of integrals of the same compound, visually locating the peaks of compounds, and identifying any overlap areas.

Results and Discussion

Accuracy

For many years, a combination of GC, HPLC, DSC (differential scanning calorimetry), and TGA (thermal gravimetric analysis, which determines the amount of volatiles in a sample) were used at this laboratory to establish the purity of a proposed reference drug standard (14). GC-MS and FT-IR were used routinely for identification, and the results were compared to the literature. NMR was used for identification only in those instances when literature spectra for MS and IR were not found or did not match.

Currently, however, all proposed reference standards are subjected to NMR quantitation. This is, in part, because NMR is the only technique that gives an absolute (not relative) purity value. In addition, impurities (if any) that are detected can also be quantitated once they are identified. (In contrast, DSC, GC (using area %), and HPLC (using area %) can only detect impurities that are observable by their respective detectors).

In over 100 proposed reference drug standards, the results of NMR, using multiple signals to determine the purity, agreed with those obtained using other authentication methods. In those cases where there was a difference, further investigation determined that the standard was not in the suspected hydrate form (for example, it was the anhydrous form instead of the monohydrate). The need to rely on an absolute quantitative method such as NMR is illustrated in the following examples.

A heroin HCl standard was synthesized in-house from reference grade morphine and submitted for authentication. Purities by DSC and GC and HPLC (area percent method) were >99%, but the NMR quantitation was 103% (multiple analyses). There were 3 possibilities why the heroin HCl purity was high by NMR: 1) the internal standard weight was less than thought, 2) the work up of the heroin HCl from heroin base was incomplete, and some heroin base was present, or 3) the sample was not the monohydrate, (the commonly found form), but instead was nearly anhydrous. The proposed standard had been run in D_2O with maleic acid. The concentration of maleic acid was checked and rechecked against known purity reference standard compounds, and eliminated possibility #1. A sample of an older authenticated heroin HCl was analyzed by NMR in $CDCl_3$, and its chemical shifts were compared to the proposed standard's. The chemical shifts were the same, meaning there was no heroin base present (Note: studies at this laboratory show that heroin base chemical shifts are very different compared to those of heroin HCl when placed in $CDCl_3$. The change in chemical shift of a heroin HCl sample as heroin base is added is almost linear with respect to molar ratio). Finally, TGA analysis determined that this proposed standard was nearly anhydrous (0.15 waters of hydration). Further investigation showed that the chemist had used a recrystallization method that prevented the incorporation of water in the standard.

In another case, reference grade MDMA Phosphate was synthesized in-house, but it was not known how many molecules of MDMA would associate with each molecule of phosphoric acid. The TGA indicated the proposed standard was anhydrous, and GC (area percent) indicated no impurities present, so the purity was expected to be at or near 100%. NMR quantitation determined the molecular weight to be that of the 1:1 drug/acid ratio by using the quantitation equation, setting purity at 100%, and solving for the molecular weight of the sample.

Determination of Mixtures of Seized Drugs and Related Compounds

The NMR spectrum of a compound will have, at most, as many peak groups to integrate as there are non-equivalent,

TABLE 1—*Reference standards, solvent, internal standard, chemical shifts suitable.*

Compound	Solvent(s)	Internal Standards*	Solubility (mg/ml)	Signals Used for Quantitation (position in ppm with number of protons). Signals in **Bold and Underlined** are Preferred if Other Numbers Present	Decomposition Rate (%/hour)
acetaminophen	D_2O	A	7.2	7.2 d(2), 6.9 d(2), 2.1 s(3)	<0.1
acetylcodeine $HCl \cdot H_2O$	D_2O	A	>24	6.9 d(1), 6.8 d(or 3 peaks)(1), 3.8 s(3)	0.1
alprazolam	$CDCl_3$	B	>19	7.7(1), 7.5(2), 7.3–7.5(5), 5.5(1), 4.1(1), 2.6(3)	<0.1
alprazolam	CD_3OD	B, C	>21	7.8 m(2), 7.5 m(3), 7.4 m(3), 5.3 d(1), 4.2 d(1), 2.6 s(3)	0.1
aminophylline	D_2O	A	5.6	7.9s(1), 3.5s(3), 3.3s(3) [a singlet at 3.15 is $NH_2CH_2CH_2NH_2$] the ethylenediamine part of this drug is more soluble than the theophylline part	<0.1
aminopyrine	$CDCl_3$	B	>24	7.4 m(4), 7.2 m(1), 2.9 s(3), 2.8 s(6), 2.2 s(3)	<0.1
aminopyrine	D_2O	A	>34	7.5–7.7 m(3), 7.3 d(2), 3.2 s(3), 2.9 s(6), 2.3 s(3) [see dipyrone, common peaks]	<0.1
amitriptyline HCl	D_2O	A	>32	7.0–7.4 m(7), 6.85 d(1), 5.75 t(1)	0.1
amphetamine sulfate	D_2O	A	>30	3.6 sextet(1), 2.9 m(2), 1.3 d(3)	<0.1
antipyrine	D_2O	A	>33	7.55–7.65 m(3), 3.25s(3), 2.3s(3) [singlet at 5.5 quickly exchanges with D_2O and disappears in about 20 hours]	<0.1
atropine sulfate	D_2O	A	>29	7.3–7.5 m(10), 5.1t(2), 4.2 m(2), 3.9–4.0 m(4), 3.8 m(2), 3.7 m(2), 2.7 s(6), 2.2–2.4 m(4), 1.5–1.6 m(2)	<0.1
aspirin	D_2O	A	2.9	8.0 d(1), 7.7 dd(1), 7.45 dd(1), 7.2 d(1), 2.35 s(3) [slowly decomposes to salicylic acid]	0.1
benzocaine	D_2O	A	4	8.1 d(2), 7.4 d(2), 4.4 q(2), 1.4 t(3)	<0.1
benzocaine	$CDCl_3$	B	>9	7.8 d(2), 6.6 d(2), 4.3 q(2), 1.4 t(3)	<0.1
benzphetamine HCl	D_2O	A	>11	[2 forms present causing doubling of peaks] 7.1–7.6 m(10), [4.2 d + 4.4 d (1)], 4.2 d + d(1), 3.7 m(1), [3.3 dd + 3.1 dd(1)], 2.9 m(1), 2.8 s + s(3), 1.3 d + d(3)	<0.1
benzphetamine HCl	CD_3OD	A	>14	7.6 m(5), 7.3 m(5), 4.6 d + d(1), 4.3 dd(1), 3.7 m(1), 2.8–2.9 t + s(4), 1.3 t(3) [2 forms present causing doubling of signals]	<0.1
1-benzylpiperazine	$CDCl_3$	B	>25	7.3(4), 3.5(2), 2.9(4), 2.4(4)	<0.1
1-benzylpiperazine base	CD_3OD	A	>21	7.2–7.7 m(5), 3.5 s(2), 3.0 m(4), 2.5 m(4) [beware: maleic acid reacts with this compound resulting in 2 maleic acid peaks. Add both peaks.]	<0.1
boldenone undecylenate	$CDCl_3$	B	>36	7.0, 6.2, 6.1	<0.1
4-bromo-2,5-dimethoxy-*beta*-phenethylamine HCl	D_2O	A	>24	7.2 s(1), 7.0 s(1), 3.8 s + s(6), 3.2 t(2), 2.9 t(2)	<0.1
bufotenine monooxalate monohydrate	CD_3OD	A	>16	7.2 d(1), 7.1 s(1), 6.9 d(1), 6.7 dd(1), 3.4 dd(2), 3.1 dd(2), 2.9 s(6)	<0.1
1,4-butanediol	D_2O	A	>20	3.6(4), 1.6(4)	<0.1
N-butylamphetamine HCl	D_2O	A	>21	7.3–7.5 m(5), 3.6 m(1), 3.0–3.2 m(2), 2.8 dd(1), 1.2 d(3)	<0.1
caffeine	D_2O	A	20	pure sample: 7.7s(1), 3.7s(3), 3.3s(3), 3.1s(3). In heroin samples with low concentration caffeine: 7.9s(1), 3.9s(3), 3.5s(3), 3.3(s)	<0.1
caffeine	$CDCl_3$	B	>8	7.5 s(1), 4.0 s(3), 3.6 s(3), 3.4 s(3)	<0.1
chloroquine diphosphate	D_2O	A	>12	8.3 d(1), 8.2 d(1), 7.8 d(1), 7.5 dd(1), 6.8 d(1), 4.1 m(1), 3.2 m(6), 1.8 m(4), 1.4 d(3), 1.25dt(6)	<0.1
chlorpheniramine maleate	D_2O	A	>25	8.6 d(1), 8.3 dt(1), 7.9 d(1), 7.7 dd(1), 7.4 d(2), 7.3 d(2), 4.4 dd(1), 2.9 s + s(6) [Beware: the maleate will increase the integral of the internal standard]	<0.1
clobenzorex HCl	D_2O	A	>11	7.7 dd(1), 7.6 dd(1), 7.5 m(2), 7.4 m(2), 7.3 m(3), 4.4 m(2), 3.6 m(1), 2.8 dd(1)	<0.1

TABLE 1—*Continued*.

Compound	Solvent(s)	Internal Standards*	Solubility (mg/ml)	Signals Used for Quantitation (position in ppm with number of protons). Signals in **Bold and Underlined** are Preferred if Other Numbers Present	Decomposition Rate (%/hour)
clobenzorex HCl	CD_3OD	A	>13	7.7 dd(1), 7.6 dd(1), 7.5 m(2), 7.4 m(2), 7.3 m(3), 4.4 m(2), 3.6 m(1), 2.8 dd(1)	<0.1
clomiphene citrate	CD_3OD	A	>16	[2 forms present] 6.7–7.5 m(14), (4.4 m + 4.2 m(2)), (3.7 m + 3.6 m(2)), 1.2–1.4 t + t(6) [citric acid 2.9 d(2), 2.8 d(2)]	<0.1
cocaine HCl	D_2O	A	>200	8.0 d(2), 7.7 t(1), 7.5 t(2), 5.6 q(1), 2.9 s(3)	<0.1
codeine phosphate hemihydrate	D_2O	A	>11	6.9 d(1), 6.8 d + small peak (1), 5.7 dm(1), 5.1 dd(1), 4.4 m(1), 3.85 s(3) [2 forms present (major and minor)]	<0.1
dextromethorphan $HBr{\cdot}H_2O$	D_2O	A	>26	7.25 (2 big peaks, 1 small)(1), 7.0 d(1), 6.9 dd(1), 3.8 s(3), 2.5 b d(1)	<0.1
diazepam	$CDCl_3$	B	>16	7.6 d(2), 7.5 m(2), 7.42 t(2), 7.3 d + s(2), **4.8 d(1) 3.8 d(1) 3.4 s(3)**	<0.1
diazepam	CD_3OD	A	9.0	7.8 d(1), 7.7 m(2), 7.6 d(1), 7.2 d(1), 4.6 d(1), 4.0 d(1), 3.4 s(3)	
diltiazem HCl	D_2O	A	>22	this is an amide and has a major and a minor form present (10:1 ratio): 7.3–8.0 m(6), 6.9 + 7.0 d + d(2), 5.0–5.4 d + d + d + d(2), 3.7–3.8 s + s(3), 2.8–3.0 s + s(6), [add integrals 1.9 s(3) + 1.4 s(3)]	0.1
3,4-dimethoxyamphetamine HCl	D_2O	A	>14	**7.0 d(1), 6.95 d(1), 6.90 dd(1), 3.7s(3), 3.7s(3)**, 3.6 sextet(1), **2.9 m(2), 1.3 d(3)**	<0.1
2,5-dimethoxy-4-N-propylthio-*beta*-phenethylamine HCl (2-CT-7)	$CDCl_3$ + CD_3OD (2 + 1)	B	>25	6.9 s(1), 6.8 s(1), 3.85 s(3), 3.8 s(3), 3.2 t(2), 3.0 t(2), 2.8 dd(2), 1.6 sextet(2), 1.0 t(3)	0.14
2,5-dimethoxy-4-iodo-*beta*-phenethylamine HCl (2-CI)	D_2O	A	>24	7.4 s(1), 6.9 s(1), 3.8 s + s(6), 3.2 t(2), 2.9 t(2)	<0.1
dimethylamphetamine HCl	D_2O	A	>26	7.3–7.5 m(5), 3.7 m(1), 3.1 dd(1), 2.8 dd + s + s(7), 1.2 d(3)	<0.1
dimethylsulfone	$CDCl_3$	B	>3	3.0s(6)	<0.1
dimethylsulfone	D_2O	A	>60	3.1 s(6)	<0.1
diphenhydramine HCl	D_2O	A	>32	7.3–7.5 m(10), 5.5 s(1), 3.7 dd(2), 3.3 dd(2), 2.8 s(6)	<0.1
dipyrone	D_2O	A	>15	3 forms present. Add integrals of peaks in brackets: 7.5–7.7 m(3), 7.4 m(2), [4.8s + 4.4s + 4.2 s], [3.3 s + 3.2 s], [3.0 s + s], [2.4 s + 2.3 s] [see aminopyrine, has common peaks]	<0.1
ephedrine HCl	D_2O	A	>33	7.3–7.5 m(5), 5.1 d(1), 3.6 m(1), 2.8s(3), 1.1 d(3)	<0.1
fenfluramine HCl	D_2O	A	>22	7.7 m(2), 7.6 m(2), 3.6 m(1), 3.1–3.3 m(3), 2.9 dd(1), 1.3 t(3), 1.2 d(3)	<0.1
fexofenadine HCl	CD_3OD	A	>12	7.5 d(4), 7.2–7.4 m(8), 7.15 t(2), 4.6 t(1), 1.4 s(6)	
N-formylamphetamine	$CDCl_3$	B	>5.5	8.1 s(1), 7.1–7.4 m(5), 4.4 septet(1), 2.7–2.9 m(2), 1.2 d(3)	<0.1
gamma-butyrolactone (GBL)	D_2O	D	>23	4.4 (2), 2.6 (2), 2.3 (2)	<0.1
gamma-hydroxybutyrate (GHB), sodium salt	D_2O	D	>20	3.6 (2), 2.2 (2), 1.8 (2)	<0.1
guaifenesin	D_2O	A	>22	**6.95–7.10 m(4)**, 4.1 m(2), 4.0 m(1), **3.85s(3), 3.75 dd(1),** 3.70 dd(1)	<0.1
heroin base	$CDCl_3$	B		6.8 d(1), 6.6 d(1), 5.6 m(1), 5.4 m(1), 5.1–5.2 m(2), 3.4 m(1), 3.1 d(1), 2.6 m(1)	<0.1
heroin $HCl{\cdot}H_2O$	D_2O	A	>40	7.0 d(1), 6.9 (3 peaks)(1) [2 forms present (major and minor)]	0.3

TABLE 1—*Continued*.

Compound	Solvent(s)	Internal Standards*	Solubility (mg/ml)	Signals Used for Quantitation (position in ppm with number of protons). Signals in **Bold and Underlined** are Preferred if Other Numbers Present	Decomposition Rate (%/hour)
hydroxyzine di HCl	D_2O	A	>23	7.4–7.7 m(9), 5.4 s(1), 3.4–3.9 m(16)	<0.1
ibuprofen	CD_3OD	A	>37	7.2 d(2), 7.1 d(2), 3.6 q(1), 2.4 d(2), 1.8 m(1), 1.4 d(3), 0.9 d(6)	<0.1
ketamine HCl	D_2O	A	>8	7.9 d(1), 7.6 m(3), 3.35 m(1), 2.6–2.7 m(2), 2.4 s(3), 2.1 m(1), 1.7–1.9 m(4)	<0.1
lidocaine HCl	D_2O	A	>21	7.15–7.3 m(3), 4.3 s(2), 3.35 q(4), 2.2 s(6), 1.38 t(6)	<0.1
loratadine	CD_3OD	A	>10	8.4 d(1), 7.8 d(1), 7.4 dd(1), 7.3 d(1), 7.2 d(1), 7.1 d(1), 4.1 q(2), 3.8 m(2), 2.9 m(2), 2.1–2.4 m(4), 1.2 t(3)	0.1
lorazepam	CD_3OD	C	19.3	**7.6 m(1), 7.4–7.56 (4)**, 7.25 d(1), **7.0 d(1), 5.0 s(1)**	<0.1
MDA HCl	D_2O	A	>35	6.88 d(1), 6.83 d(1), 6.78 dd(1), 6.0 s(2), 3.6 (sextet)(1), 2.8 dd + dd(2), 1.3 d(3)	<0.1
MDEA HCl	D_2O	A	>23	6.7–6.9 (3), 6.0 (2), 3.5 (1), 3.0–3.2 (3), 2.8 (1), 1.2–1.3 (6)	
MDMA HCl	D_2O	A	>30	6.7–6.9 (m)(3), 6.0 (s)(2), 3.5 (sextet)(1), 3.0 dd(1), 2.8 dd(1), 2.7 (s)(3), 1.3 (d)(3)	<0.1
meperidine HCl	D_2O	A	>11	7.35–7.6 m(5), [4.2q + 4.1q](2), [3.6dm + 3.5dm](2), [2.4dt + 2.1dt](2), [1.2t + 1.1t](3) [2 forms exist in solution creating duplicates of all signals]	<0.1
meprobamate	CD_3OD	A	>29	3.8 (m)(4), 1.3 (m)(4), 0.9 m(6). Beware: A (over time 2nd peak appears, integrate both peaks)	<0.1
methamphetamine HCl	D_2O	A	>107	3.5 m(1), 3.1 dd(1), 2.9 dd(1), 2.7(3), 1.3(3)	<0.1
methamphetamine HCl	$CDCl_3$	B	>58	3.5 m(1), 3.1 dd(1), 2.9 dd(1), 2.7(3), 1.3(3)	<0.1
methamphetamine HCl	CD_3OD	A	>16	7.3 m(3), 3.5 m(1), 3.2 dd(1), 2.8 dd + s(4), 1.2 d(3)	<0.1
methandrostenolone	$CDCl_3$	B	>25	7.0 (d)(1), 6.2 (d)(1), 6.0 (s)(1), 0.9 (s)(3)	<0.1
methandrostenolone	CD_3OD	C	>19	7.3 d91), 6.2 d(1), 6.0 s(1), 2.6 dt(1), 2.4 dt(1), 2.0 m(1), 1.5–1.9 m(7), 0.95–1.4 m(11), 0.90 s(3)	<0.1
methaqualone HCl	D_2O	A	5.7	8.4(1), 8.1(1), 7.8(2), 7.6(2), 7.5(1), 7.4(1), 2.2(3). Singlet at 2.6(3) decreases over time due to exchange of methyl with deuterium from solvent	1.0
5-methoxy-*alpha*-methyltryptamine HCl	D_2O	A	>21	7.5 d(1), 7.3s(1), 7.2 d(1), 6.95 dd(1), 3.9s(3), 3.7 m(1), 3.0–3.2 m(2), 1.4 d(3)	<0.1
5-methoxy-NN-dimethyltryptamine base	$CDCl_3$	B	>10	7.2 d(1), 7.05 m(1), 6.95 m(1), 6.8 dd(1), 3.85 s(3), 2.9 m(2), 2.6 m(2), 2.35 s(6)	<0.1
methylphenidate	D_2O	A	>20	7.4–7.5 m(3), 7.3 m(2), 4.0 d(1), 3.8 dt (1), 3.7 s(3), 3.45 d m(1), 3.1 dt(1), 1.75–1.95 m(2), 1.55–1.7 m(2), 1.3–1.5 m(2)	<0.1
methyl salicylate	D_2O	A	0.6	7.9 dd(1), 6.56 ddd(1), 7.0 m(2)	<0.1
methyl salicylate	D_2O	A	0.6	7.9 dd(1), 6.56 ddd(1), 7.0 m(2), 3.9 s(3)	<0.1
morphine HCl ($3\text{-}H_2O$)	D_2O	A	>6.0	6.8(1), 6.7(1), 5.7 (1), 5.1 (1), 4.4 (1)	<0.1
morphine sulfate (2:1) pentahydrate	CD_3OD	A	5	6.65 d(2), 6.55 d(2), 5.8 m(2), 5.3 m(2), 4.3 m(2), 4.2 m(2)	<0.1
O6-MAM HCl ($2\text{-}H_2O$)	D_2O	A	8.6	6.8 (d)(1), 6.7 (d or 3 peaks)(1), 4.2 (m)(1)	
O6-MAM base	$CDCl_3$	B	>5	6.65 d(1), 6.5 d(1), 5.05 d(1), 3.4 m(1), 3.0 d(1), 2.7 m(1), 2.2s(3)	<0.1
N-methyl-*alpha*-benzyl-*beta*-phenethylamine HCl	D_2O	A	>21	7.2–7.5 (m)(10), 3.8 (pentet)(1), 2.9 + 3.2 (dd,dd)(4), 2.7 (s)(3)	<0.1

TABLE 1—*Continued.*

Compound	Solvent(s)	Internal Standards*	Solubility (mg/ml)	Signals Used for Quantitation (position in ppm with number of protons). Signals in **Bold and Underlined** are Preferred if Other Numbers Present	Decomposition Rate (%/hour)
naproxen sodium	$CDCl_3$+ CD_3OD (1 + 1)	B	>7	**7.6–7.8 m(3),** 7.5 dd(1), **7.0–7.2 d + dd(2), 3.9 s(3)**, 3.7 q(1), **1.5 d(3)**	<0.1
noscapine base	D_2O	A	6.5	7.5b d(1), 6.55 s(1), 5.9 s(1), 5.8 bs(1), 5.3 s(1), 3.92 s(3), 3.85 s(3)	1.0
noscapine base	$CDCl_3$	B	>9	6.95 d(1), 6.3 s(1), 6.1 d(1), 5.95 dd(2), 5.6 d(1), 4.4 d(1), 4.1 s(3), 4.0 s(3), 3.8 s(3), 2.6 m(1), 2.55 s(3), 2.35 m(2), 1.9 m(1)	<0.1
oxandrolone	$CDCl_3$	B	>14	4.2 d(1), 3.9 d(1), 2.5 dd(1), 2.2 dd(1), 1.0 s(3), 0.7–0.95 s + m(5)	<0.1
oxandrolone	CD_3OD	C		4.2 d(1), 3.9 d(1), 2.5 dd(1), 2.2 dd(1), 1.0 s(3), 0.7–0.95 s + m(5)	
oxazepam	$CDCl_3$	B	2.5	7.6 d(2), 7.5 m(2), 7.42 t(2), 7.3 d(1), 7.1 d(1), 5.0 s(1)	<0.1
oxazepam	$CDCl_3$/ CD_3OD (2 + 0.5)	B	>10	7.5–7.6 m(4), 7.4–7.5 m(2), 7.3 d(1), 7.2 d(1), 4.9 s(1)	<0.1
oxycodone HCl monohydrate	D_2O	A	>11	7.0 dd*(1), 6.9 dd*(1), 3.9ss*(3)*= major and minor form (tautomers) peaks integrated	<0.1
oxymetholone	$CDCl_3$	B, C	>24	8.6 s(1), 2.4 d(1), 2.3 dd(1), 1.9–2.1 dd + d(2), 0.9 (s + m)(4), 0.7–0.8 (s + m)(4) BE CAUTIOUS OF 8.6 PPM SIGNAL	0.1
papaverine base	$CDCl_3$	B	>8	8.4 d(1), 7.4 d(1), 7.3 s(1), 7.0 s(1), 5.8 m(2), 4.5 s(2), 4.0 s(3), 3.9 s(3), 3.8 s(3), 3.75 s(3)	<0.1
papaverine HCl (anhydrous)	D_2O	A	4.4	8.2 d(1), 8.0 d(1), 7.6 s(1), 7.5 s(1), 7.1 d(1), 7.0 d(1), 6.9 dd(1), 4.1 s(3), 4.0 s(3), 3.8 s(3), 3.8 s(3) [2 singlets at 3.8 sometimes combine as one singlet]	<0.1
phenacetin	D_2O	A	0.8	7.4 d(2), 7.0 d(2), 4.1 q(2), 2.1 s(3), 1.3 t(3)	<0.1
phenacetin	$CDCl_3$	B	>7	**7.4 d(2)**, 6.8 d(2), 4.0 q(2), **2.1 s(3)**, 1.4 t(3)	<0.1
phencyclidine HCl (PCP)	D_2O	A	10	7.5–7.7 m(5), 3.7 b d(2), 3.0 b d(2), 2.4 bt(4)	0.4
phenmetrazine HCl	D_2O	A	>27	7.5 m(5), 4.5 d(1), 4.2 m(1), 4.0 m(1), 3.6 m(1), 3.4 m(2), 1.1 d(3)	<0.1
phenobarbital	D_2O	A	0.8	7.4 m(5), 2.5q(2), 1.0t(3)	<0.1
phenobarbital	CD_3OD/ D_2O (1:1)	A	>20	7.4 (m)(5), 2.4 (q)(2), 1.0 (t)(3)	<0.1
phenobarbital	$CDCl_3$	B	>4	7.4 m(5), 2.5 q(2), 1.0 t(3)	<0.1
phentermine HCl	D_2O	A	>16	7.4 m(3), 7.3 d(2), 3.0s(2), 1.4s(6)	<0.1
phenylacetone (P2P)	$CDCl_3$	B	>25	7.34 (2), 7.20 (2), 3.7 (2), 2.2 (3)	<0.1
phenylpropanolamine HCl (PPA)	D_2O	A	>24	7.4–7.5 m(5), 5.0 d(1), 3.7 m(1), 1.2 d(3)	<0.1
piperazine base	D_2O	A	>15	3.0 s(8) [beware: piperazine reacts with maleic acid slowly over time. Add peaks at 6.0 and 6.5 PPM for maleic acid integral]	<0.1
procaine HCl	D_2O	A	>33	in heroin: 8.2 d(2), 7.4 d(2), 1.4t(6)—pure procaine HCl: 7.9 d(2), 7.0 d(2), 4.6 m(2), 3.6 m(2), 3.4q(2), 1.4t(6)	<0.1
propoxyphene HCl	D_2O	A	>18	7.3–7.5 m(8), 7.2 d(2), 3.8 m(2), 3.4 d(1), 2.8 s(3), 2.7 m + s(4), 2.6 m(1), 2.4 m(2), 1.0–1.1d + t(6)	<0.1
pseudoephedrine HCl	D_2O	A	>28	7.4 m(5), 4.7 d(1), 3.5 m(1), 2.75 s(3), 1.1 d(3)	<0.1
rofecoxib	$CDCl_3$	B	>12	7.9 d(2), 7.5 d(2), 7.4 m(5), 5.2 s(2), 3.1 s(3)	<0.1
salicylic acid	D_2O	A	1.3	7.9 dd(1), 7.56d dd(1), 7.0 dd(1)	<0.1
secobarbital	$CDCl_3$	B	>11	5.6m, 5.1(d + d)(2), 2.8 m(2), 2.2 m(1), 1.5 m(2)	<0.1
sildenafil citrate (Viagra®)	CD_3OD	A	>21	8.2 d(1), 8.0 dd(1), 7.4 d(1), 4.3 q(2), 4.2 s(3), 1.8 m(2), 1.5 t(3), 1.0 t(3) [citric acid: 2.8 d(2)]	<0.1

TABLE 1—*Continued*.

Compound	Solvent(s)	Internal Standards*	Solubility (mg/ml)	Signals Used for Quantitation (position in ppm with number of protons). Signals in **Bold and Underlined** are Preferred if Other Numbers Present	Decomposition Rate (%/hour)
stanazolol	CD_3OD	C	>5	2.6 d + dd(2), 2.2 dd(1), 2.1 d(1), 0.9 s(3) [do not use peak at 7.2]	<0.1
stanazolol	CD_3OD	A	6	7.4 s(1), 2.6 d + dd(2), 2.3 dd(1), 2.1 d(1), 1.2 s(3), 0.9–1.0 m + s(5), 0.85 s(3)	<0.1
tadalafil (Cialis®)	$CDCl_3$	B	>13	7.6 d(1), 7.15 m(2), 6.8 d(1), 6.7 s + d(2), 6.1 s(1), 5.8 d(2), 4.3 m(1), 4.1 d(1), 3.9 d(1), 3.8 dd(1), 3.2 dd(1), 3.0 s(3)	<0.1
tamoxifen citrate	D_2O	A	>14	7.3–7.6 m(10), 7.1 d(2), 6.9 d(2), 4.4 dd(2), 3.8 dd(2), 2.7 q(2)	<0.1
thiamine HCl	D_2O	A	>18	8.0 s(1), 5.6 s(2), 3.9t(2), 3.2t(2), 2.6 s(3), 2.5 s(3) [there is a singlet at 9.6 but it exchanges with D_2O at 0.7%/hr)	<0.1
1-(3-trifluoromethyl) phenylpiperazine	$CDCl_3$	B	>25	7.4 (1), 7.0–7.2 (3), 3.2 (4), 3.0 (4)	<0.1
2,4,5-trimethoxy-amphetamine base	$CDCl_3$ + CD_3OD (2 + 1)	B	>22	6.9 d(1), 6.7 d(1), 3.9 s(3), 3.8 s(6), 3.5sextet(1), 2.9 dd(1), 2.8 dd(1), 1.2 d(3)	<0.1
tripelennamine HCl	D_2O	A	>30	8.1 d dd(1), 7.8 d dd(1), 7.2–7.5 m(5), 6.9–7.0 m(2), 4.0 t(2), 3.4 t(2), 2.9 s(6)	<0.1
triprolidine HCl monohydrate	D_2O	A	>20	**8.6 dd(1), 8.3 dd(1), 7.8 m(2), 7.4 d(2), 7.2 d(2), 6.7 t(1), 4.1 d(2), 2.4 s(3)** [wide multiplets that are less accurate: 3.7 m(2), 3.0 m(2), 1.9–2.1 m(4)]	<0.1
xylazine HCl	D_2O	A	>22	7.2–7.4 m(3), 2.1–2.3 m(8), [must combine the following signals due to 2 forms in existance: {3.6t + 3.4dd (2)}, {3.3dd + 3.1t (2)}]	<0.1
zolpidem base	D_2O + CD_3OD (1 + 0.5)	A	9	**8.2 s(1), 7.8 m(2), 7.4 m(4), 3.2 s(3), 3.0 s(3), 2.5 s(3), 2.4 s(3)** [do not use 4.3 s(2)]	<0.1
zolpidem hemitartrate	D_2O	A	>13	**8.2 s(1), 7.8 d(1), 7.7 d(1), 7.4 m(4),** [tartaric acid: 4.7 s(1)], **3.2 s(3), 3.0 s(3), 2.5 s(3), 2.3 s(3)** [do not use 4.3 s(2)]	<0.1

* A = Maleic Acid and TMS or TSP.
B = Methenamine and TMS.
C = Dimethylsulfone and TMS.
D = Dioxane and TSP.

non-exchangeable hydrogens. Because no separation takes place in NMR, the more compounds that are present in the sample, the more chances for overlap of signals. However, with high field magnets, high resolution shimming systems, deuterium gradient shimming, and advances in methods of integrating peaks, purities of even structurally similar molecules in the same sample can be accurately determined, often with at least one "clean" single compound integral.

For example, methamphetamine dissolved in D_2O (with maleic acid as an internal standard) contains 6 groups of peaks that are baseline resolved (Fig. 1). Common impurities that can be present in a methamphetamine sample include ephedrine and pseudoephedrine, but these have only the phenyl region (7.2–7.5 PPM) in common with methamphetamine, and so are easily differentiated and quantified. A complex illicit methamphetamine sample containing 7 drugs, 1 sugar, and some water insoluble material (starch) is shown in Figs. 2*a–e*. At least one clean integral for each compound was found, and by subtracting the contributions of various compounds from mixed integrals, the following quantitative results with standard deviations were obtained: methamphetamine (2 clean integrals, 5.4+/−0.3% as the HCl), pseudoephedrine (3 results, 3.3+/−0.0% as the HCl), ephedrine (3 results, 0.5+/−0.1% as the HCl), ketamine (3 results, 27.4+/−0.5% as the HCl), chloroquine (7 results, 12.8+/−0.6% as the diphosphate), chlorpheniramine (3 results, 1.7+/−0.3% as the maleate), caffeine (2 results, 6.9+/−0.3%), and lactose. To be conservative, the lowest quantitative value is reported. It was determined that the chlorpheniramine was the maleate form because the maleic acid to TSP integral ratio was higher than expected, indicating that an interfering signal was present under the maleic acid peak; that is, from the maleate form of chlorpheniramine. The maleic acid to TSP ratio was used to correct the quantitative results.

This example is not a typical methamphetamine sample. The majority of methamphetamine samples submitted to our lab are usually free flowing powders that are either methamphetamine HCl with trace amounts of ephedrine and/or pseudoephedrine HCl, or methamphetamine HCl cut with dimethylsulfone (Fig. 3). Dimethylsulfone is a compound without a chromophore, making it invisible by UV detection. By quantitating the methamphetamine HCl, dimethylsulfone, ephedrine HCl, and pseudoephedrine HCl, total results of 95–100% are common, with water and solvents accounting for the remainder of the sample. Dry samples totaling less than 95% are often determined, using microscopy, to contain inorganics (e.g., sodium chloride), starches, or cellulose.

Heroin samples are more complex to elucidate due to the carryover of opium alkaloids and their acetylated counterparts from the

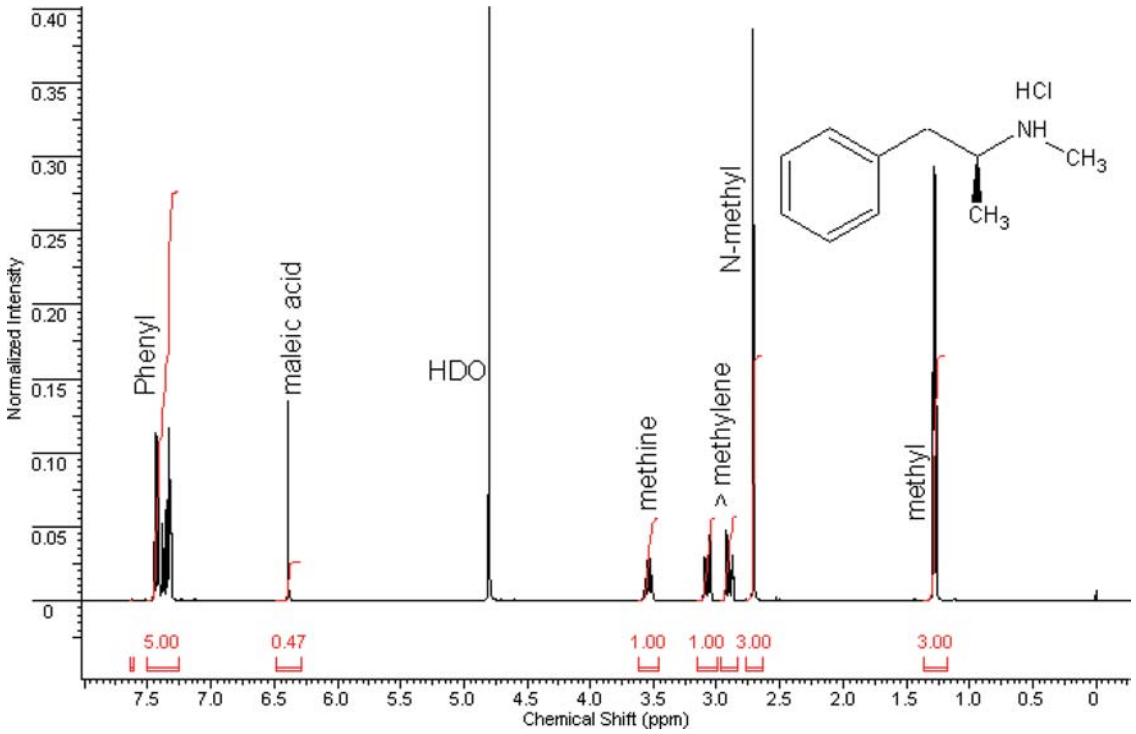


FIG. 1—*Spectrum and structure of methamphetamine HCl in D_2O containing maleic acid (internal standard) and TSP (0 PPM reference). Integrals under methamphetamine peaks indicate the number of protons present at that chemical shift.*

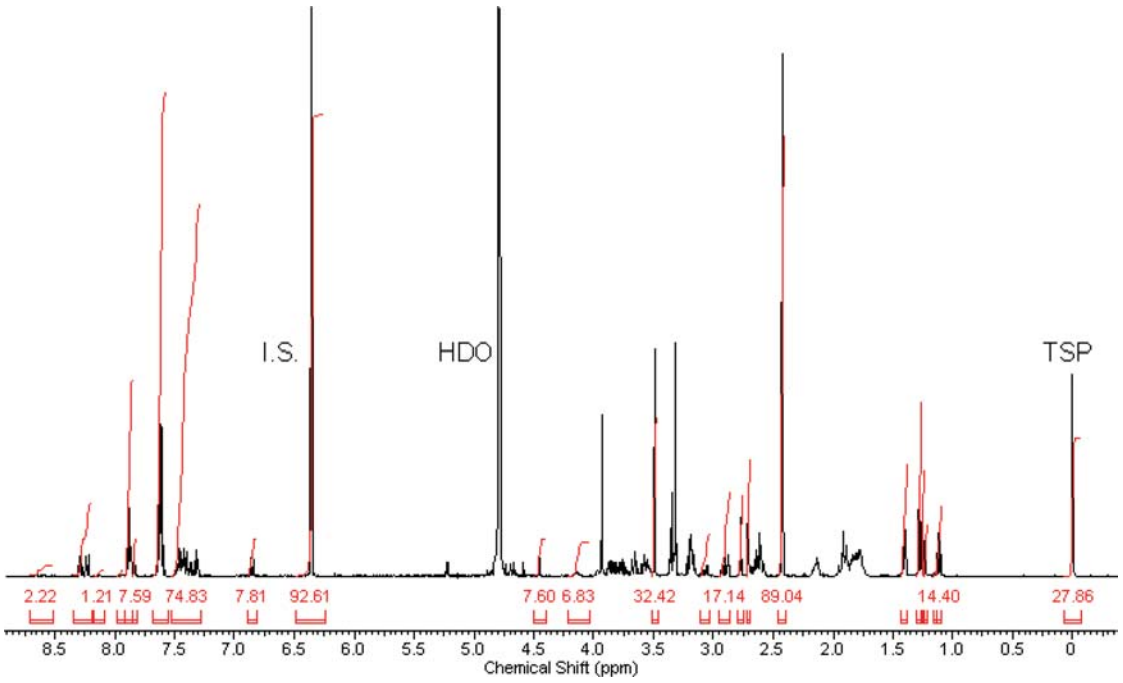
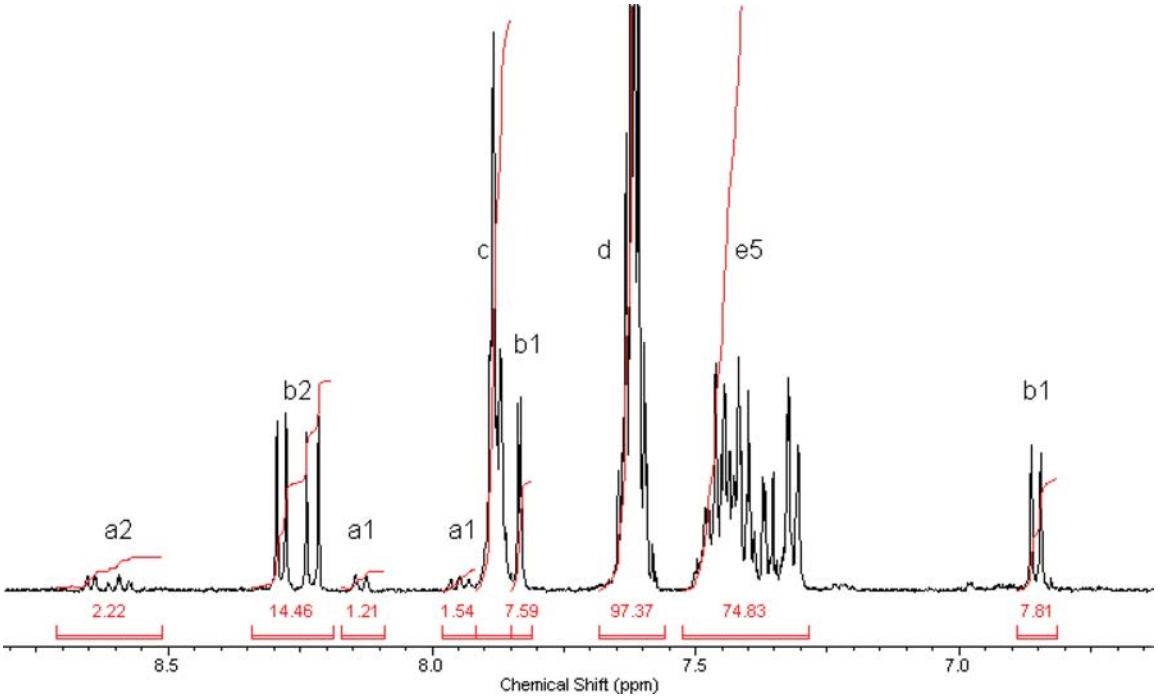


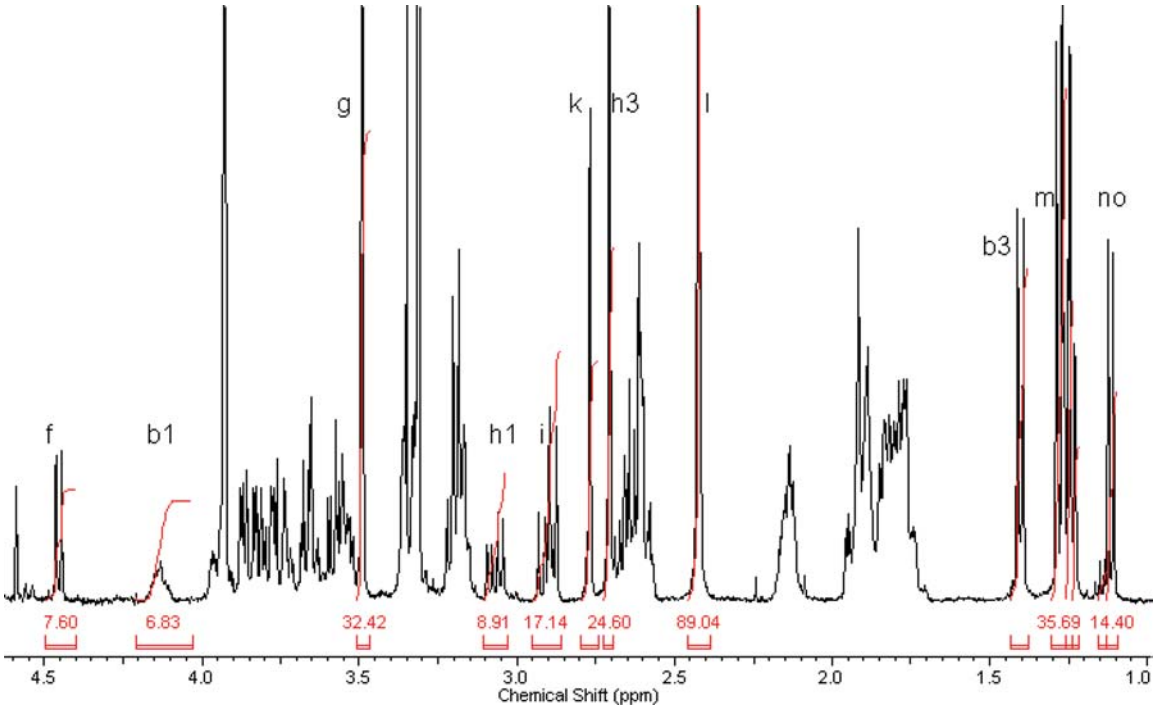
FIG. 2*a*—*Full spectrum of an unusually complex exhibit containing methamphetamine HCl (5.1%), chlorpheniramine maleate (1.5%), chloroquine diphosphate (11.9%), caffeine (7.1%), ephedrine HCl (0.4%), pseudoephedrine HCl (3.3%), ketamine HCl (26.9%), and lactose (8.8%). Numbers to the right of the letter indicate number of hydrogens for that compound at that location. Methamphetamine had 5 signals to use, chloroquine 7, ketamine 3, caffeine 2, ephedrine 2, pseudoephedrine 2, and lactose 1. The maleic acid/TSP ratio indicated the chlorpheniramine was the maleate.*

illicit synthesis process. Codeine, morphine, O6-acetylcodeine, O3- and O6-monoacetylmorphine (MAM) have many overlapping signals. However, with the exceptions of O3-MAM and papaverine, the heroin signals at 6.9 and 7.0 PPM are isolated, and can be used for quantitation. Accurate quantitation is possible because O3-MAM (usually in trace amounts) and papaverine have other signals at other independent locations, whose integrals can be determined and then subtracted from both the heroin integrals. There are a few adulterants that can also interfere with the 2 heroin signals, such as salicylic acid, acetaminophen, and phenacetin, but their contribution to the heroin integral can also be similarly subtracted out.



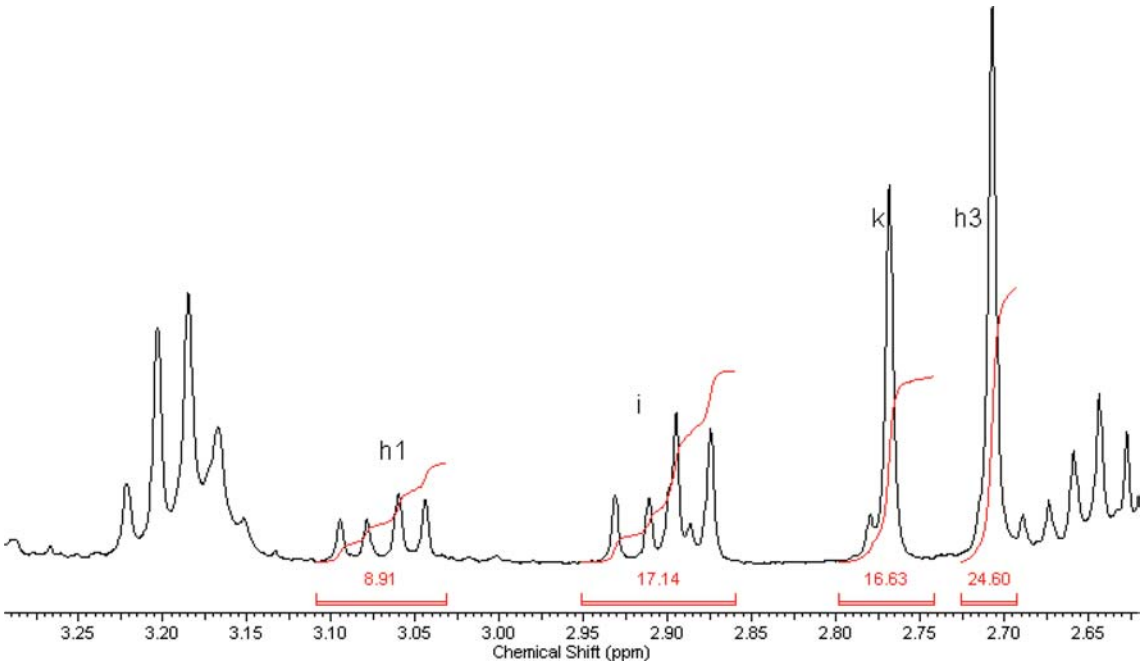
a = chlorpheniramine, b = chloroquine, c = ketamine (1) + caffeine (1) d = ketamine (3) + chloroquine (1), e = methamphetamine (5) + pseudoephedrine (5) + ephedrine (5)

FIG. 2*b*—*Aromatic region of spectrum.*



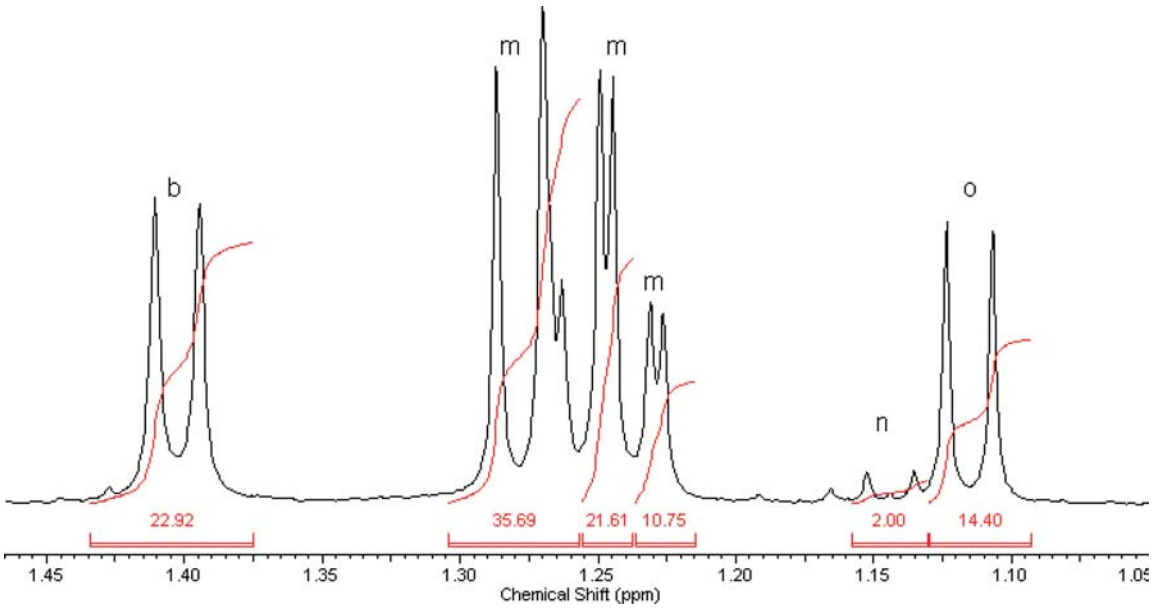
b = chloroquine, f = lactose (1), g = caffeine (3), h = methamphetamine, i = methamphetamine (1) + chlorpheniramine (1), k = pseudoephedrine (3) + ephedrine (3), l = ketamine (3), m = chloroquine + methamphetamine, n = ephedrine (3), 0 = pseudoephedrine (3)

FIG. 2*c*—*Upfield region of the spectrum.*



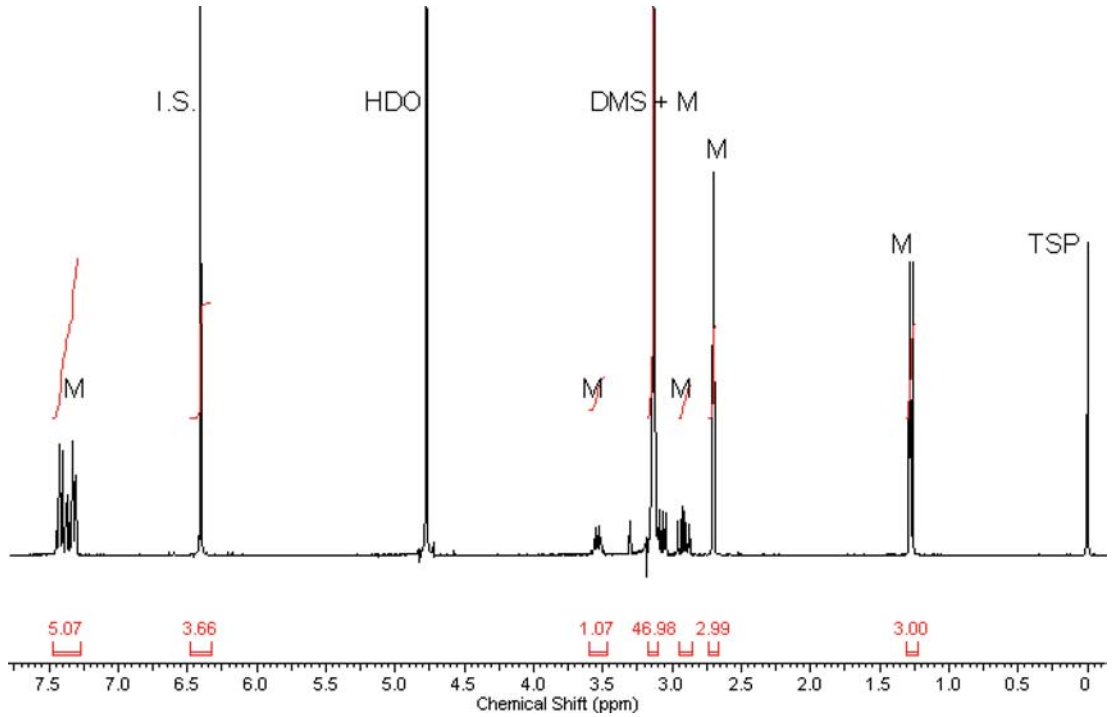
h = methamphetamine, i = methamphetamine (1) + chlorpheniramine (6), k = ephedrine (3) + pseudoephedrine (3)

FIG. 2*d*—*Focus on methamphetamine methylene peaks (3.07 and 2.90 PPM) and N-methyl peak (2.70 PPM). The large and small singlets at 2.8 PPM are ephedrine (smaller peak) and pseudoephedrine. Peak height of ephedrine versus sum of peak heights can be used to determine the percentage of the integral is ephedrine.*



b = chloroquine (3), m = methamphetamine (3) + chloroquine [left integral methamphetamine (3) + chloroquine (1.5), middle integral chloroquine (3), right integral chloroquine (1.5)], m = ephedrine (3), n = pseudoephedrine (3)

FIG. 2*e*—*The methamphetamine quantitation for the doublet at 1.28 PPM can be derived 2 ways. Integrate the entire region 1.2-1.3 PPM (contains methamphetamine and 6 hydrogens of chloroquine) and subtract twice the chloroquine integral at 1.4 PPM (representing 3 hydrogens) (68.05−2 ∗ 22.92 = 22.21 or 4.6%) or integrate as shown and subtract the right 2 peaks at 1.22 PPM from the integral at 1.28 PPM (35.69−10.75 = 24.94 or 5.2%).*



I.S. = maleic acid, HDO = deuterium oxide, DMS = dimethylsulfone, M = methamphetamine HCl, TSP = 0 PPM reference

FIG. 3—*Illicit methamphetamine HCl (20%) cut with dimethylsulfone (80%) in D_2O with maleic acid as internal standard.*

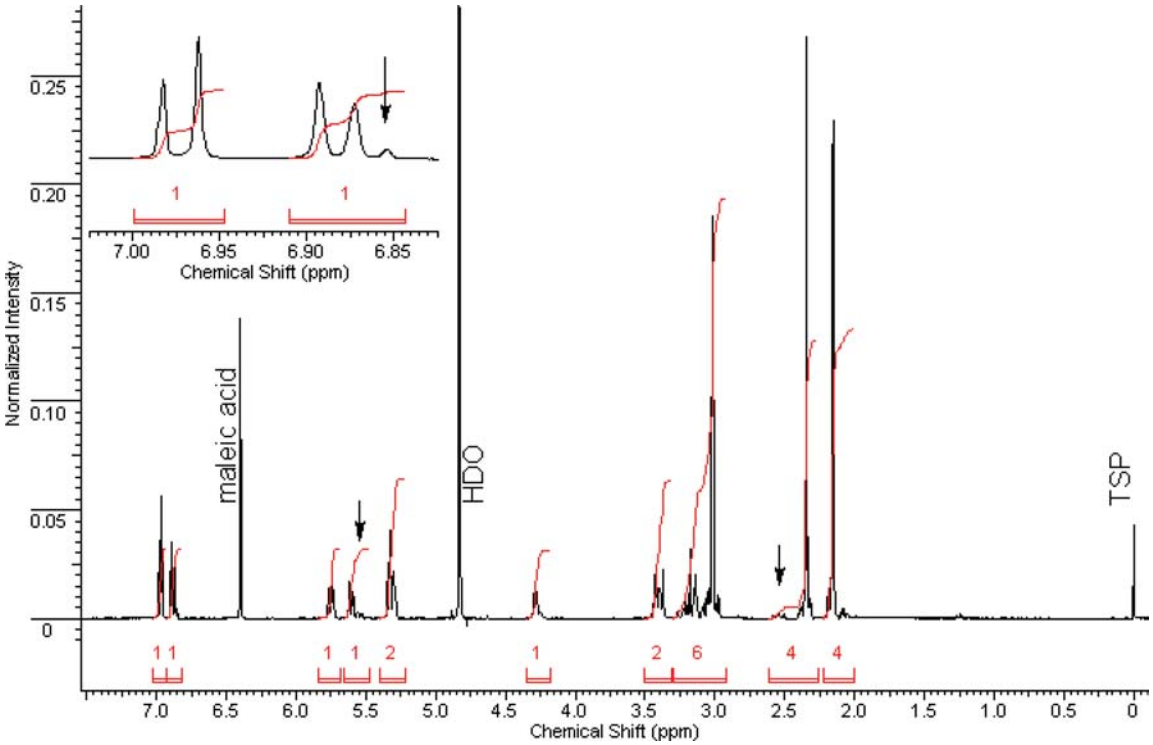
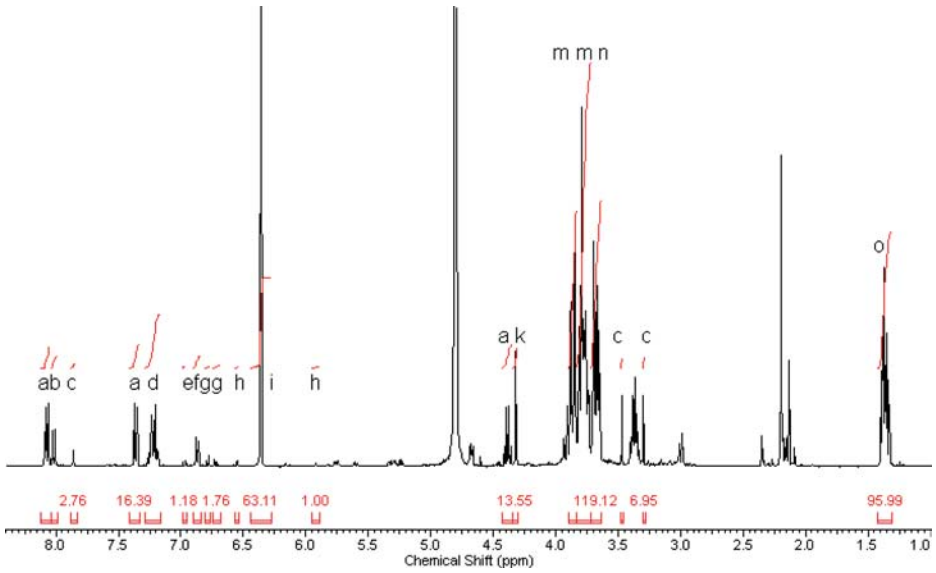


FIG. 4—*Heroin HCl (reference standard) in D_2O with maleic acid as internal standard and TSP as reference. Arrows show where minor form of heroin has a different chemical shift from the major form. Signals below 6 PPM are common for many of the morphine/codeine compounds.*

Of note for all morphine/codeine type compounds, the HCl ion-pair (not the base) in D_2O with maleic acid indicates the presence of a major and a minor form (Fig. 4 for heroin HCl). This is due to the formation of two different ion-pair conformations (see arrows in Fig. 4). However, the base forms of these compounds show only one form. Other compounds exhibiting multiple forms are benzphetamine HCl, diltiazem HCl, dipyrone, meperidine HCl, oxycodone HCl (a tautomer), and xylazine HCl.



a = benzocaine (2), b = procaine (2) c = caffeine (1),
d = lidocaine (3) + procaine (2) + acetamineophen (2), e = heroin (1),
f = heroin (1) + acetaminophen (2), g = O6-monoacetylmorphine (1), h = moscapine (1),
i = internal standard (maleic acid (2)), k = lidocaine (2), m = mannitol (2,4),
n = mannitol (2) and procaine (2), o = benzocaine (3), lidocaine (6), procaine (6)

FIG. 5*a—Exhibit containing heroin HCl (2.5%), benzocaine (6.6%), procaine HCl (6.5%), lidocaine HCl (9.1%), acetaminophen (2.8%), caffeine (2.2%), O6-monoacetylmorphine HCl (3.8%), noscapine base (2.0%), and mannitol (48.5%). At least one clean signal found for all compounds except acetaminophen, whose purity was derived by subtracting contribution of heroin to a mix of heroin and acetaminophen.*

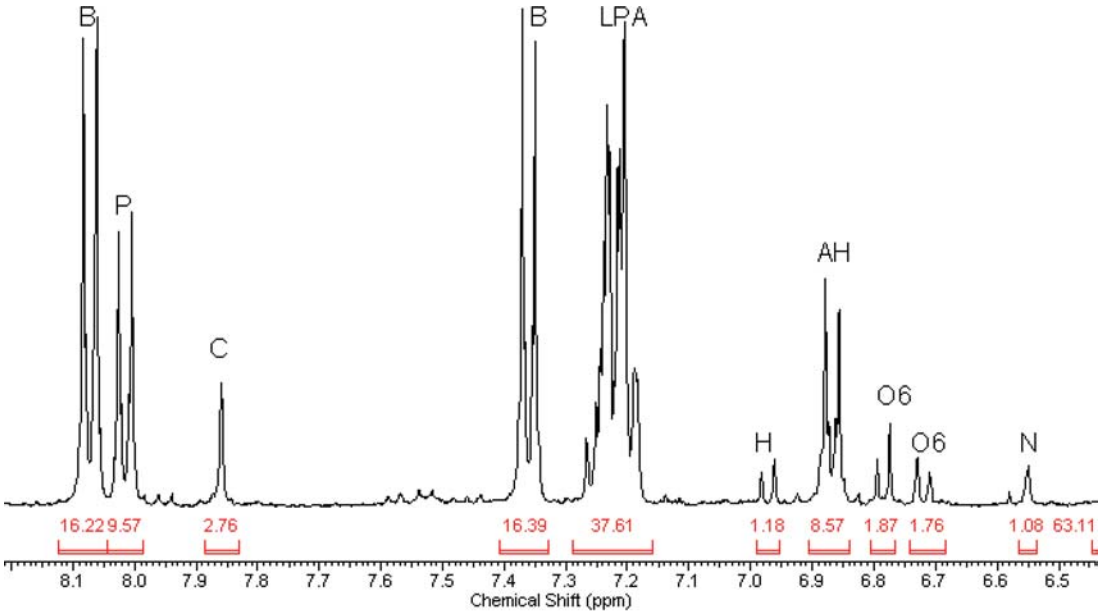


FIG. 5*b—Aromatic region of spectrum of illicit heroin. By subtracting the heroin integral at 6.95 PPM (1.18) from the heroin-acetaminophen integral at 6.85 PPM (8.57) the acetaminophen integral can be determined (7.39).*

Figures 5*a–c* show sections of a busy spectrum of a cut heroin sample in D_2O (with maleic acid as the internal standard). Every compound except acetaminophen has at least one signal free of interferences. By subtracting the clean heroin integral from the acetaminophen-heroin integral ($8.57-1.18=7.39$), a quantitative result of 2.8% acetaminophen is obtained. By subtracting the contributions of procaine (2 hydrogens, 9.57) and lidocaine (3 hydrogens, 20.33) from the integral at 7.2 PPM ($37.61-9.57-20.33=7.72$), a

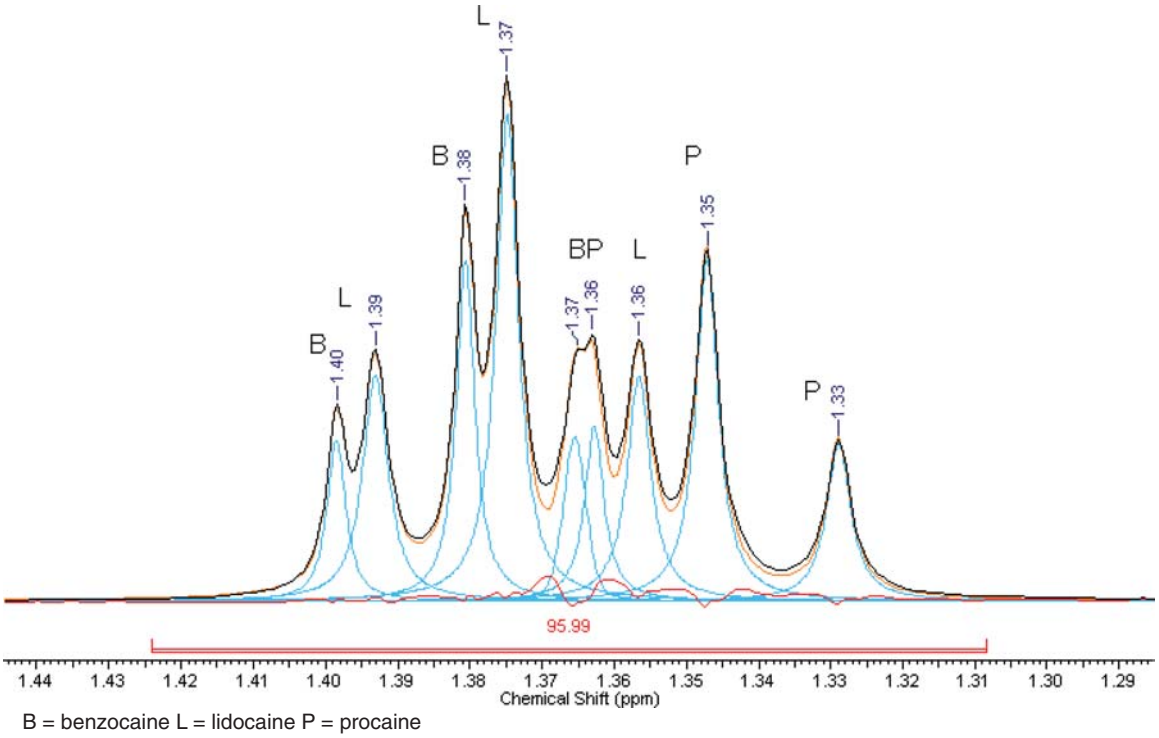


FIG. 5*c*—*Deconvolution of the triplets of lidocaine, benzocaine, and procaine can determine areas of these peaks. Peaks under the spectrum are calculated individual peaks. Line at the bottom is the residual of spectrum and individual calculated peaks.*

result of 2.9% for acetaminophen is obtained, agreeing with the first result. If required, deconvolution could be used to determine the areas of a mixture of the 3 triplets at 1.3 PPM for benzocaine, procaine, and lidocaine (Fig. 5*c*). Deconvolution determines the individual peak curves (shown under the spectrum) which, when added together, make the spectrum. The wavy horizontal line at the bottom represents the difference between the experimental spectrum and the calculated peaks; that is, the residual spectrum. The flatter the residual line, the better the fit. Quantitative results determined from deconvolution for benzocaine (6.6%), lidocaine HCl (9.6%), and procaine HCl (6.4%) agree with the other determinations. This sample contained heroin HCl (1 clean integral, 2.5%), benzocaine (4 results, 6.7 +/− 0.1%), procaine HCl (2 results, 6.6 +/− 0.1%), lidocaine HCl (2 results, 9.4 +/− 0.4%), acetaminophen (2 results, 2.9 +/− 0.1%), caffeine (3 results, 2.4 +/− 0.3%), O6-MAM HCl (2 results, 3.9 +/− 0.1%), noscapine (2 results, 2.1 +/− 0.1% as base), and mannitol (not quantitated). CE (13) results for this sample were heroin HCl (2.2%), O6-MAM HCl (3.6%), and noscapine (as base, 2.0%), comparing favorably with those of NMR.

When incorporated in plastics, heroin (base and HCl) can be analyzed by NMR using $CDCl_3$ containing methenamine and TMS as the internal standards. Results by NMR have agreed well with those obtained by GC but with 2 added benefits. The first was that NMR could detect the presence of the siloxane compounds (peaks located at approximately 0.1 PPM) that are sometimes found in plastics (and that will stay on a GC column for a considerable time, coming off as broad humps over the course of several injections). The second is that the base or acid ion-pair can be determined using the chemical shifts of the heroin. If the ion-pair is present, further tests such as silver nitrate are used to determine which acid is present.

Figure 6 shows the spectrum of MDMA HCl reference standard in D_2O with maleic acid as the internal standard. Figures 7*a–d* show the spectrum for a tablet containing MDMA HCl (4 clean integrals, 23.8 +/− 0.3%), methamphetamine HCl (3 clean integrals, 3.9 +/− 0.0%), acetaminophen (2 clean integrals, 33.5 +/− 0.1%), caffeine (2 clean integrals, 3.0 +/− 0.1%), and sucrose. Results using deconvolution on the methyl signals (Fig. 7*d*) were 23.7% and 23.3% for MDMA HCl, and 3.4% and 3.8% for methamphetamine HCl at 2.7 and 1.3 PPM, respectively.

Figure 8 shows cocaine HCl standard in D_2O with maleic acid. Figure 9 shows a portion of a spectrum of an illicit seizure containing cocaine HCl at 79.5%, *cis*-cinnamoylcocaine HCl at 1.6%, *trans*-cinnamoylcocaine HCl at 2.3% (3.9% combined), and hydroxyzine HCl at 7.2% by NMR. GC quantitation for these compounds was determined as 77.3% cocaine HCl and 3.3% combined *cis*- and *trans*-cinnamoylcocaine HCl. Hydroxyzine is not detectable by GC unless derivatized.

There does come a point at which the complexity of the sample is too great for quick NMR quantitation, but this tends to be rare in bulk drug seizures. What can be a more serious limiting factor to NMR quantitation are samples that contain substances (i.e., metal containing complexes, rust, salts) that cause the T2 (spin-spin relaxation) value to be short, resulting in a FID of less than a second. This causes peak widths to broaden to greater than 1 Hz, lowering resolution. Using other solvents, such as $CDCl_3$, can prevent these substances from going into solution, and thereby greatly improve resolution. However, this may pose solubility problems for the

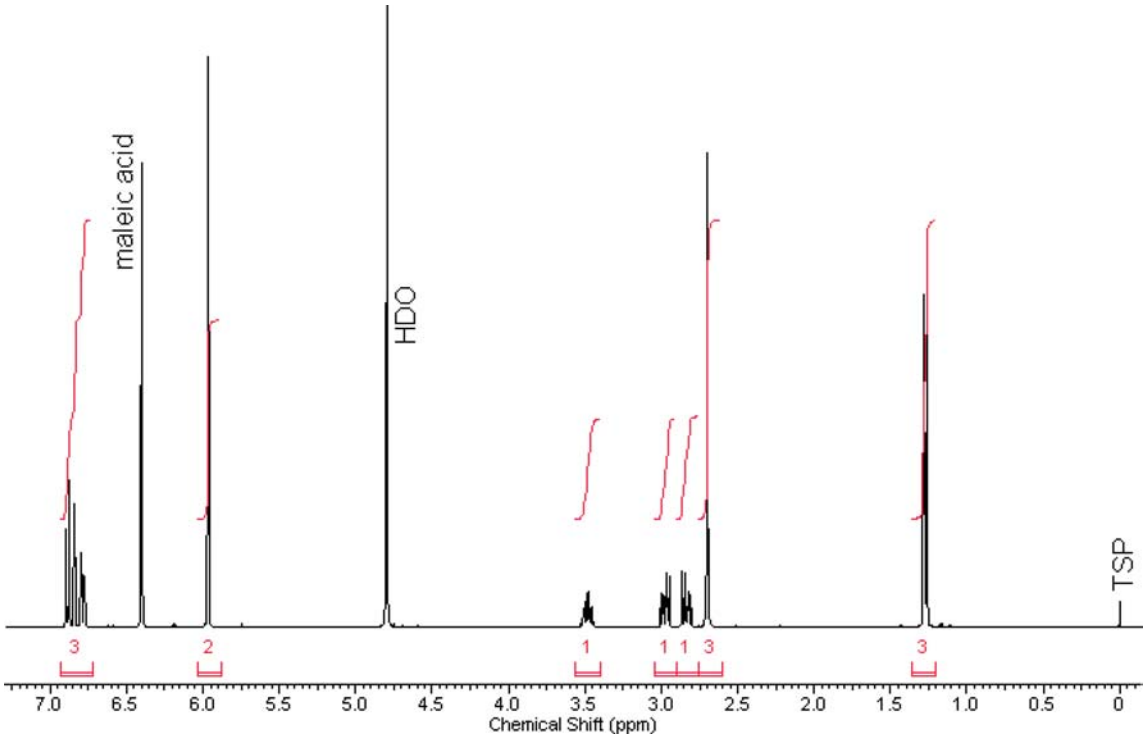
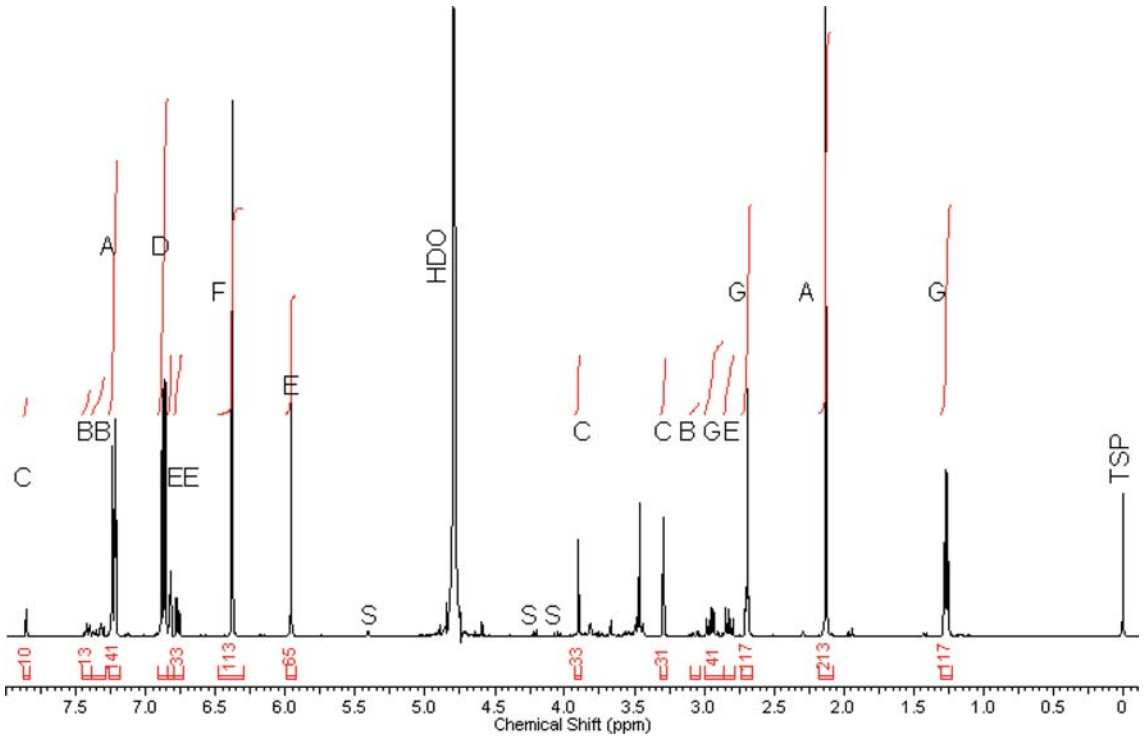
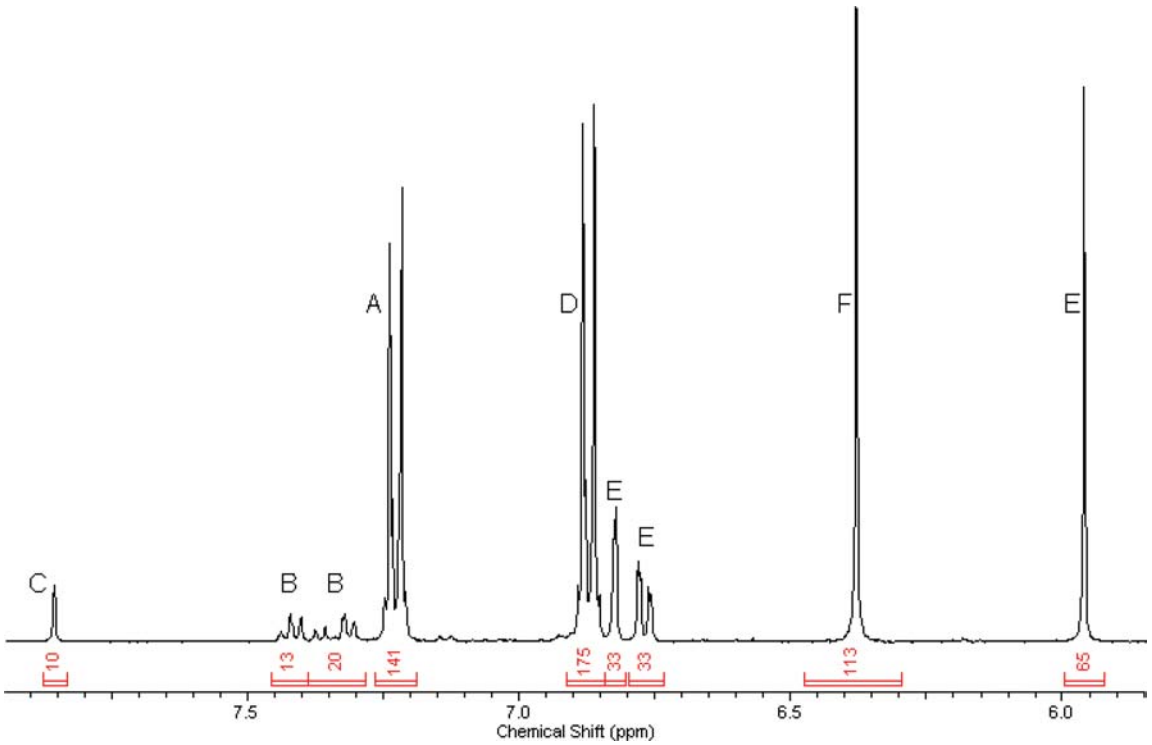


FIG. 6—*MDMA HCl reference standard in D_2O with maleic acid. Integrals show number of hydrogens at each group of peaks.*



A = acetaminophen, B = methamphetamine, C = caffeine, D = acetaminophen + MDMA, E = MDMA,
F = maleic acid (I.S.), G = MDMA + methamphetamine, S = sucrose

FIG. 7*a*—*MDMA HCl (23.8%), methamphetamine HCl (3.9%), caffeine (3.0%), acetaminophen (33.5%), and sucrose in D_2O with maleic acid as internal standard and TSP as 0 PPM reference.*



A = acetaminophen, B = methamphetamine, C = caffeine, D = acetaminophen + MDMA, E = MDMA, F = maleic acid (I.S.)

FIG. 7*b—Aromatic region of sample. All signals except "D" are free of interferences.*

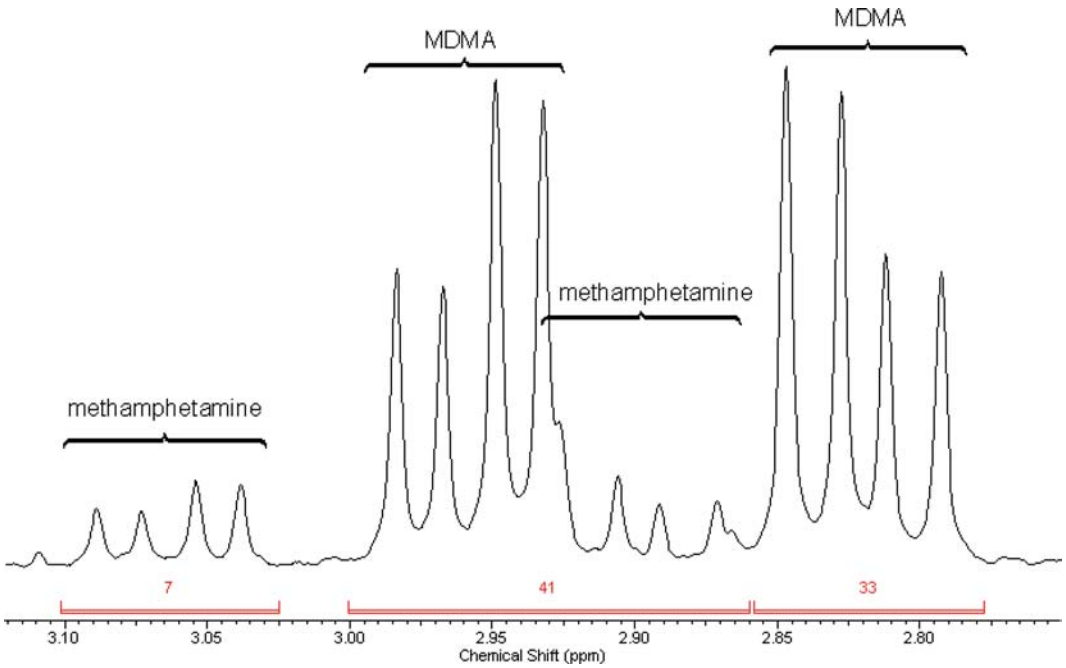


FIG. 7*c—Methylene hydrogen doublet of doublets for MDMA and methamphetamine.*

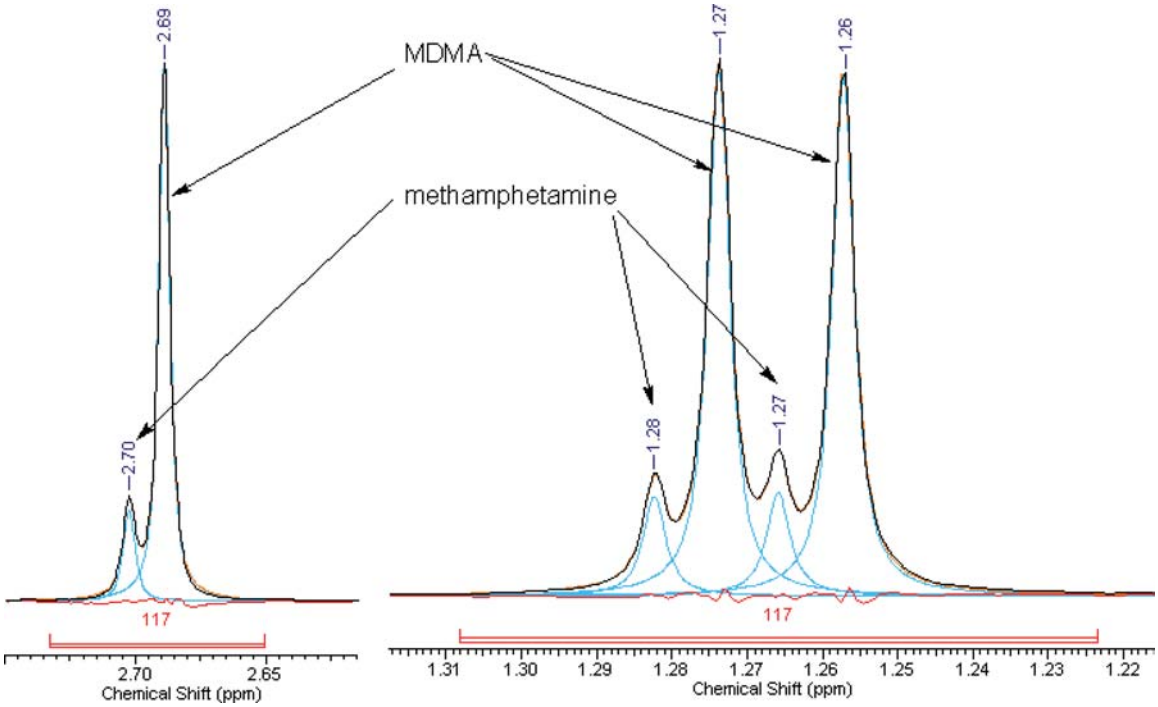


FIG. 7*d—Deconvolution of methyl groups for MDMA and methamphetamine. Molar fraction of MDMA determined as 0.850 and 0.832, and methamphetamine as 0.150 and 0.168, at 2.7 and 1.3 PPM, respectively. MDMA HCl calculated as 23.7% and 23.3%, and methamphetamine HCl calculated as 3.4% and 3.8%, at 2.7 PPM and 1.3 PPM, respectively.*

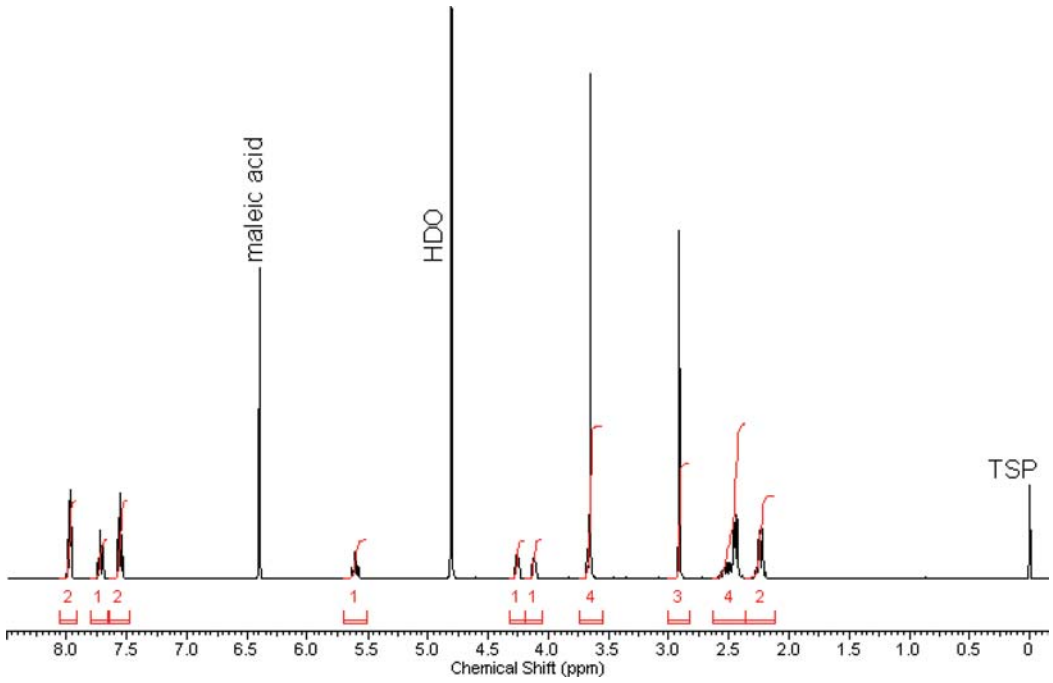
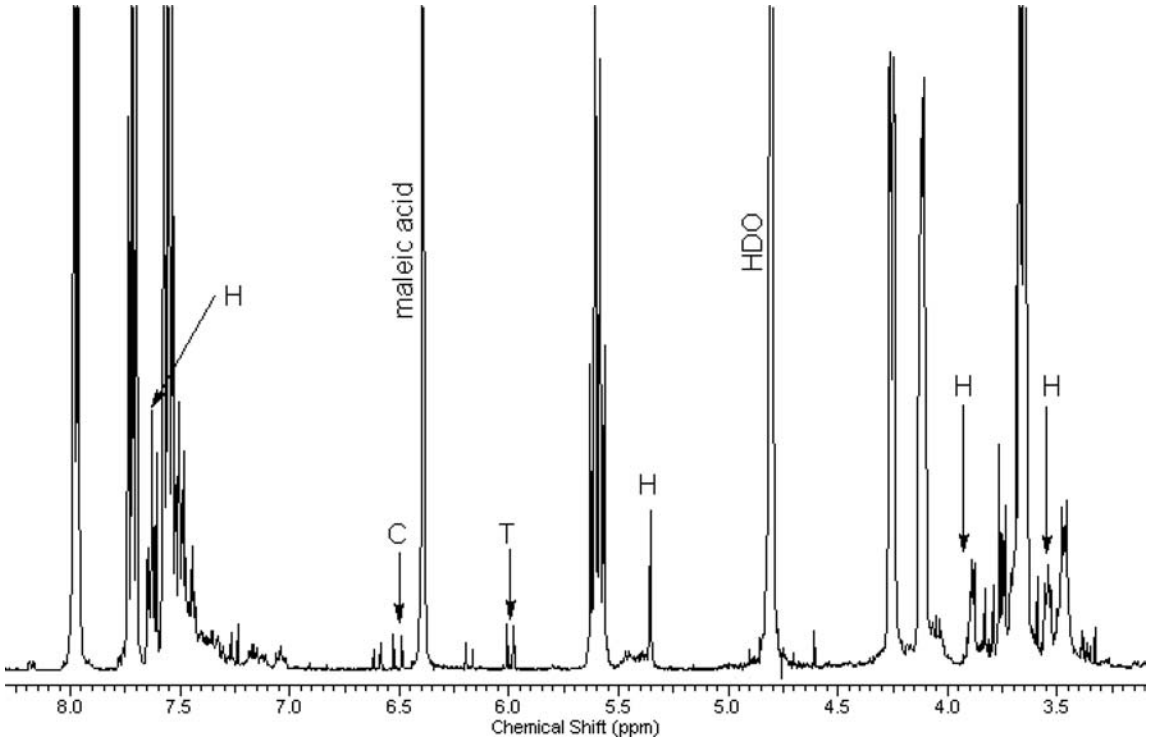


FIG. 8—*Cocaine HCl reference standard in D_2O with maleic acid. Integrals reflect the number of hydrogens at that chemical shift.*



C = cis-cinnamoylcocaine, T = trans-cinnamoylcocaine, H = hydroxyzine

FIG. 9—*Illicit cocaine HCl containing cis- and trans-cinnamoylcocaine and hydroxyzine HCl. Vertical scale adjusted to view the minor components.*

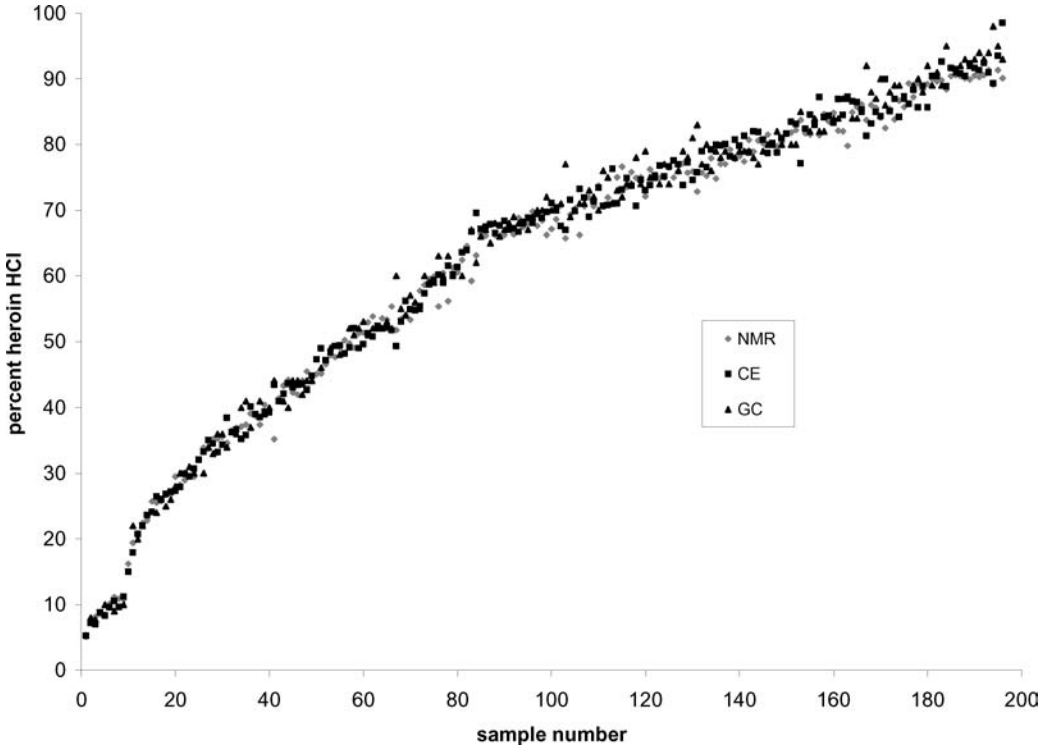


FIG. 10—*196 heroin samples analyzed by NMR, CE, and GC showing good agreement between methods.*

target drug(s). Dilution of the sample can help to lengthen the FID and improve resolution, but this lowers S/N. Should the S/N be less than 10:1, then acquiring 32 scans instead of 8 will double the S/N, but the experiment time will be longer.

Comparisons with Other Methods

A random sampling of 196 heroin exhibits, ranging in heroin HCl values from 5–98%, were analyzed by NMR, GC, and CE (Fig. 10), and showed good agreement. These samples came from

bulk seizures of heroin that were first analyzed at a DEA regional laboratory using GC, with a representative sample sent to our laboratory. The samples were analyzed by NMR to confirm heroin purity, determine the presence and identity of any sugars, and quantitate and identify adulterants.

Comparing the results of NMR to CE and GC, a linear relationship was found with slopes of 1.0085 and 1.0167, respectively. These curves go through the origin, and correlation coefficients of 0.9892 and 0.9846, respectively, were obtained. Of the 196 samples examined, 25 had RSDs of >5%, where 14 of the GC results were out of agreement with NMR and CE, 5 NMR results were out of agreement with GC and CE, and 4 CE results were out of agreement with GC and NMR. The large number of GC outliers is probably due to sample inhomogeneity in some large seizures, while the CE and NMR analyses were performed on a smaller homogeneous sample weeks and sometimes months older than the GC sample. Some heroin seizures were uncut, while others contained several adulterants and sugars. Adulterants found and quantitated by NMR included caffeine, lidocaine, acetaminophen, procaine, chloroquine, quinine, diphenhydramine, theophylline, aspirin, phenobarbital, cocaine, salicylic acid, phenacetin, creatine, benzocaine, and aminopyrine. In the case of heroin with chloroquine, the GCs used in our laboratory cannot resolve these compounds, but their NMR spectra are well resolved, as are their peaks in CE (13).

Analyses of 21 exhibits of illicit methamphetamine HCl by GC or HPLC at another DEA laboratory were compared to NMR at this laboratory, and showed purities of 23–100%, with percent difference (versus NMR) ≤6.8%.

Analysis of 32 exhibits of illicit cocaine HCl by GC and NMR at this laboratory determined purities of 8–91%, with percent difference (versus NMR) ≤6.7% for all but one low purity result (8.1% by NMR and 9.0% by GC).

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